of the host cells to be used for expression, that is operatively-linked to the nucleic acid sequence to be expressed. Within a recombinant expression vector, "operably-linked" is intended to mean that the nucleotide sequence of interest is linked to the regulatory sequence(s) in a manner that allows for expression of the nucleotide sequence (*e.g.*, in an *in vitro* transcription/translation system or in a host cell when the vector is introduced into the host cell).

The term "regulatory sequence" is intended to includes promoters, enhancers and other expression control elements (*e.g.*, polyadenylation signals). Such regulatory sequences are described, for example, in Goeddel, GENE EXPRESSION TECHNOLOGY: METHODS IN ENZYMOLOGY 185, Academic Press, San Diego, Calif. (1990). Regulatory sequences include those that direct constitutive expression of a nucleotide sequence in many types of host cell and those that direct expression of the nucleotide sequence only in certain host cells (*e.g.*, tissue-specific regulatory sequences). It will be appreciated by those skilled in the art that the design of the expression vector can depend on such factors as the choice of the host cell to be transformed, the level of expression of protein desired, etc. The expression vectors of the invention can be introduced into host cells to thereby produce proteins or peptides, including fusion proteins or peptides, encoded by nucleic acids as described herein (*e.g.*, FCTRX proteins, mutant forms of FCTRX proteins, fusion proteins, etc.).

The recombinant expression vectors of the invention can be designed for expression of FCTRX proteins in prokaryotic or eukaryotic cells. For example, FCTRX proteins can be expressed in bacterial cells such as *Escherichia coli*, insect cells (using baculovirus expression vectors) yeast cells or mammalian cells. Suitable host cells are discussed further in Goeddel, GENE EXPRESSION TECHNOLOGY: METHODS IN ENZYMOLOGY 185, Academic Press, San Diego, Calif. (1990). Alternatively, the recombinant expression vector can be transcribed and translated *in vitro*, for example using T7 promoter regulatory sequences and T7 polymerase.

Expression of proteins in prokaryotes is most often carried out in *Escherichia coli* with vectors containing constitutive or inducible promoters directing the expression of either fusion or non-fusion proteins. Fusion vectors add a number of amino acids to a protein encoded therein, usually to the amino terminus of the recombinant protein. Such fusion vectors typically serve three purposes: (*i*) to increase expression of recombinant protein; (*ii*) to increase the solubility of the recombinant protein; and (*iii*) to aid in the purification of the recombinant protein by acting as a ligand in affinity purification. Often, in fusion expression vectors, a proteolytic cleavage site is introduced at the junction of the fusion moiety and the recombinant protein to enable separation of the recombinant protein from the fusion moiety subsequent to purification of the fusion protein. Such enzymes, and their cognate recognition sequences, include Factor Xa,

thrombin and enterokinase. Typical fusion expression vectors include pGEX (Pharmacia Biotech Inc; Smith and Johnson, 1988. *Gene* 67: 31-40), pMAL (New England Biolabs, Beverly, Mass.) and pRIT5 (Pharmacia, Piscataway, N.J.) that fuse glutathione S-transferase (GST), maltose E binding protein, or protein A, respectively, to the target recombinant protein.

Examples of suitable inducible non-fusion *E. coli* expression vectors include pTrc (Amrann *et al.*, (1988) *Gene* 69:301-315) and pET 11d (Studier *et al.*, GENE EXPRESSION TECHNOLOGY: METHODS IN ENZYMOLOGY 185, Academic Press, San Diego, Calif. (1990) 60-89).

One strategy to maximize recombinant protein expression in *E. coli* is to express the protein in a host bacteria with an impaired capacity to proteolytically cleave the recombinant protein. *See, e.g.,* Gottesman, GENE EXPRESSION TECHNOLOGY: METHODS IN ENZYMOLOGY 185, Academic Press, San Diego, Calif. (1990) 119-128. Another strategy is to alter the nucleic acid sequence of the nucleic acid to be inserted into an expression vector so that the individual codons for each amino acid are those preferentially utilized in *E. coli* (*see, e.g.,* Wada, *et al.*, 1992. *Nucl. Acids Res.* 20: 2111-2118). Such alteration of nucleic acid sequences of the invention can be carried out by standard DNA synthesis techniques.

In another embodiment, the FCTRX expression vector is a yeast expression vector. Examples of vectors for expression in yeast *Saccharomyces cerivisae* include pYepSec1 (Baldari, *et al.*, 1987. *EMBO J.* 6: 229-234), pMFa (Kurjan and Herskowitz, 1982. *Cell* 30: 933-943), pJRY88 (Schultz *et al.*, 1987. *Gene* 54: 113-123), pYES2 (Invitrogen Corporation, San Diego, Calif.), and picZ (InVitrogen Corp, San Diego, Calif.).

Alternatively, FCTRX can be expressed in insect cells using baculovirus expression vectors. Baculovirus vectors available for expression of proteins in cultured insect cells (*e.g.*, SF9 cells) include the pAc series (Smith, *et al.*, 1983. *Mol. Cell. Biol.* 3: 2156-2165) and the pVL series (Lucklow and Summers, 1989. *Virology* 170: 31-39).

In yet another embodiment, a nucleic acid of the invention is expressed in mammalian cells using a mammalian expression vector. Examples of mammalian expression vectors include pCDM8 (Seed, 1987. *Nature* 329: 840) and pMT2PC (Kaufman, *et al.*, 1987. *EMBO J.* 6: 187-195). When used in mammalian cells, the expression vector's control functions are often provided by viral regulatory elements. For example, commonly used promoters are derived from polyoma, adenovirus 2, cytomegalovirus, and simian virus 40. For other suitable expression systems for both prokaryotic and eukaryotic cells see, *e.g.*, Chapters 16 and 17 of Sambrook, *et al.*, MOLECULAR CLONING: A LABORATORY MANUAL. 2nd ed., Cold Spring Harbor Laboratory, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1989.

In another embodiment, the recombinant mammalian expression vector is capable of directing expression of the nucleic acid preferentially in a particular cell type (*e.g.*, tissue-specific regulatory elements are used to express the nucleic acid). Tissue-specific regulatory elements are known in the art. Non-limiting examples of suitable tissue-specific promoters include the albumin promoter (liver-specific; Pinkert, *et al.*, 1987. *Genes Dev.* 1: 268-277), lymphoid-specific promoters (Calame and Eaton, 1988. *Adv. Immunol.* 43: 235-275), in particular promoters of T cell receptors (Winoto and Baltimore, 1989. *EMBO J.* 8: 729-733) and immunoglobulins (Banerji, *et al.*, 1983. *Cell* 33: 729-740; Queen and Baltimore, 1983. *Cell* 33: 741-748), neuron-specific promoters (*e.g.*, the neurofilament promoter; Byrne and Ruddle, 1989. *Proc. Natl. Acad. Sci. USA* 86: 5473-5477), pancreas-specific promoters (Edlund, *et al.*, 1985. *Science* 230: 912-916), and mammary gland-specific promoters (*e.g.*, milk whey promoter; U.S. Pat. No. 4,873,316 and European Application Publication No. 264,166). Developmentally-regulated promoters are also encompassed, *e.g.*, the murine hox promoters (Kessel and Gruss, 1990. *Science* 249: 374-379) and the α-fetoprotein promoter (Campes and Tilghman, 1989. *Genes Dev.* 3: 537-546).

The invention further provides a recombinant expression vector comprising a DNA molecule of the invention cloned into the expression vector in an antisense orientation. That is, the DNA molecule is operatively-linked to a regulatory sequence in a manner that allows for expression (by transcription of the DNA molecule) of an RNA molecule that is antisense to FCTRX mRNA. Regulatory sequences operatively linked to a nucleic acid cloned in the antisense orientation can be chosen that direct the continuous expression of the antisense RNA molecule in a variety of cell types, for instance viral promoters and/or enhancers, or regulatory sequences can be chosen that direct constitutive, tissue specific or cell type specific expression of antisense RNA. The antisense expression vector can be in the form of a recombinant plasmid, phagemid or attenuated virus in which antisense nucleic acids are produced under the control of a high efficiency regulatory region, the activity of which can be determined by the cell type into which the vector is introduced. For a discussion of the regulation of gene expression using antisense genes *see, e.g.,* Weintraub, *et al.*, "Antisense RNA as a molecular tool for genetic analysis," *Reviews-Trends in Genetics*, Vol. 1(1) 1986.

Another aspect of the invention pertains to host cells into which a recombinant expression vector of the invention has been introduced. The terms "host cell" and "recombinant host cell" are used interchangeably herein. It is understood that such terms refer not only to the particular subject cell but also to the progeny or potential progeny of such a cell. Because certain modifications may occur in succeeding generations due to either mutation or environmental

influences, such progeny may not, in fact, be identical to the parent cell, but are still included within the scope of the term as used herein.

A host cell can be any prokaryotic or eukaryotic cell. For example, FCTRX protein can be expressed in bacterial cells such as *E. coli*, insect cells, yeast or mammalian cells (such as Chinese hamster ovary cells (CHO) or COS cells). Other suitable host cells are known to those skilled in the art.

Vector DNA can be introduced into prokaryotic or eukaryotic cells via conventional transformation or transfection techniques. As used herein, the terms "transformation" and "transfection" are intended to refer to a variety of art-recognized techniques for introducing foreign nucleic acid (*e.g.*, DNA) into a host cell, including calcium phosphate or calcium chloride co-precipitation, DEAE-dextran-mediated transfection, lipofection, or electroporation. Suitable methods for transforming or transfecting host cells can be found in Sambrook, *et al.* (MOLECULAR CLONING: A LABORATORY MANUAL. 2nd ed., Cold Spring Harbor Laboratory, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1989), and other laboratory manuals.

For stable transfection of mammalian cells, it is known that, depending upon the expression vector and transfection technique used, only a small fraction of cells may integrate the foreign DNA into their genome. In order to identify and select these integrants, a gene that encodes a selectable marker (*e.g.*, resistance to antibiotics) is generally introduced into the host cells along with the gene of interest. Various selectable markers include those that confer resistance to drugs, such as G418, hygromycin and methotrexate. Nucleic acid encoding a selectable marker can be introduced into a host cell on the same vector as that encoding FCTRX or can be introduced on a separate vector. Cells stably transfected with the introduced nucleic acid can be identified by drug selection (*e.g.*, cells that have incorporated the selectable marker gene will survive, while the other cells die).

A host cell of the invention, such as a prokaryotic or eukaryotic host cell in culture, can be used to produce (*i.e.*, express) FCTRX protein. Accordingly, the invention further provides methods for producing FCTRX protein using the host cells of the invention. In one embodiment, the method comprises culturing the host cell of invention (into which a recombinant expression vector encoding FCTRX protein has been introduced) in a suitable medium such that FCTRX protein is produced. In another embodiment, the method further comprises isolating FCTRX protein from the medium or the host cell.

Transgenic FCTRX Animals

The host cells of the invention can also be used to produce non-human transgenic animals. For example, in one embodiment, a host cell of the invention is a fertilized oocyte or an embryonic stem cell into which FCTRX protein-coding sequences have been introduced. Such host cells can then be used to create non-human transgenic animals in which exogenous FCTRX sequences have been introduced into their genome or homologous recombinant animals in which endogenous FCTRX sequences have been altered. Such animals are useful for studying the function and/or activity of FCTRX protein and for identifying and/or evaluating modulators of FCTRX protein activity. As used herein, a "transgenic animal" is a non-human animal, preferably a mammal, more preferably a rodent such as a rat or mouse, in which one or more of the cells of the animal includes a transgene. Other examples of transgenic animals include non-human primates, sheep, dogs, cows, goats, chickens, amphibians, etc. A transgene is exogenous DNA that is integrated into the genome of a cell from which a transgenic animal develops and that remains in the genome of the mature animal, thereby directing the expression of an encoded gene product in one or more cell types or tissues of the transgenic animal. As used herein, a "homologous recombinant animal" is a non-human animal, preferably a mammal, more preferably a mouse, in which an endogenous FCTRX gene has been altered by homologous recombination between the endogenous gene and an exogenous DNA molecule introduced into a cell of the animal, *e.g.*, an embryonic cell of the animal, prior to development of the animal.

A transgenic animal of the invention can be created by introducing FCTRX-encoding nucleic acid into the male pronuclei of a fertilized oocyte (*e.g.*, by microinjection, retroviral infection) and allowing the oocyte to develop in a pseudopregnant female foster animal. The human FCTRX cDNA sequences of SEQ ID NOS:1, 3, 5, 7, 9, 10, 11, 12, 14, 16, 18, 20, 22, and 24, can be introduced as a transgene into the genome of a non-human animal. Alternatively, a non-human homologue of the human FCTRX gene, such as a mouse FCTRX gene, can be isolated based on hybridization to the human FCTRX cDNA (described further *supra*) and used as a transgene. Intronic sequences and polyadenylation signals can also be included in the transgene to increase the efficiency of expression of the transgene. A tissue-specific regulatory sequence(s) can be operably-linked to the FCTRX transgene to direct expression of FCTRX protein to particular cells. Methods for generating transgenic animals via embryo manipulation and microinjection, particularly animals such as mice, have become conventional in the art and are described, for example, in U.S. Patent Nos. 4,736,866; 4,870,009; and 4,873,191; and Hogan, 1986. In: MANIPULATING THE MOUSE EMBRYO, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. Similar methods are used for production of other transgenic animals. A

transgenic founder animal can be identified based upon the presence of the FCTRX transgene in its genome and/or expression of FCTRX mRNA in tissues or cells of the animals. A transgenic founder animal can then be used to breed additional animals carrying the transgene. Moreover, transgenic animals carrying a transgene-encoding FCTRX protein can further be bred to other transgenic animals carrying other transgenes.

To create a homologous recombinant animal, a vector is prepared which contains at least a portion of an FCTRX gene into which a deletion, addition or substitution has been introduced to thereby alter, *e.g.*, functionally disrupt, the FCTRX gene. The FCTRX gene can be a human gene (*e.g.*, the cDNA of SEQ ID NOS:1, 3, 5, 7, 9, 10, 11, 12, 14, 16, 18, 20, 22, and 24), but more preferably, is a non-human homologue of a human FCTRX gene. For example, a mouse homologue of human FCTRX gene of SEQ ID NOS:1, 3, 5, 7, 9, 10, 11, 12, 14, 16, 18, 20, 22, and 24, can be used to construct a homologous recombination vector suitable for altering an endogenous FCTRX gene in the mouse genome. In one embodiment, the vector is designed such that, upon homologous recombination, the endogenous FCTRX gene is functionally disrupted (*i.e.*, no longer encodes a functional protein; also referred to as a "knock out" vector).

Alternatively, the vector can be designed such that, upon homologous recombination, the endogenous FCTRX gene is mutated or otherwise altered but still encodes functional protein (*e.g.*, the upstream regulatory region can be altered to thereby alter the expression of the endogenous FCTRX protein). In the homologous recombination vector, the altered portion of the FCTRX gene is flanked at its 5'- and 3'-termini by additional nucleic acid of the FCTRX gene to allow for homologous recombination to occur between the exogenous FCTRX gene carried by the vector and an endogenous FCTRX gene in an embryonic stem cell. The additional flanking FCTRX nucleic acid is of sufficient length for successful homologous recombination with the endogenous gene. Typically, several kilobases of flanking DNA (both at the 5'- and 3'-termini) are included in the vector. *See, e.g.,* Thomas, *et al.*, 1987. *Cell* 51: 503 for a description of homologous recombination vectors. The vector is ten introduced into an embryonic stem cell line (*e.g.*, by electroporation) and cells in which the introduced FCTRX gene has homologously-recombined with the endogenous FCTRX gene are selected. *See, e.g.*, Li, *et al.*, 1992. *Cell* 69: 915.

The selected cells are then injected into a blastocyst of an animal (*e.g.*, a mouse) to form aggregation chimeras. *See, e.g.*, Bradley, 1987. In: TERATOCARCINOMAS AND EMBRYONIC STEM CELLS: A PRACTICAL APPROACH, Robertson, ed. IRL, Oxford, pp. 113-152. A chimeric embryo can then be implanted into a suitable pseudopregnant female foster animal and the embryo brought to term. Progeny harboring the homologously-recombined DNA in their germ cells can

be used to breed animals in which all cells of the animal contain the homologously-recombined DNA by germline transmission of the transgene. Methods for constructing homologous recombination vectors and homologous recombinant animals are described further in Bradley, 1991. *Curr. Opin. Biotechnol.* 2: 823-829; PCT International Publication Nos.: WO 90/11354; WO 91/01140; WO 92/0968; and WO 93/04169.

In another embodiment, transgenic non-humans animals can be produced that contain selected systems that allow for regulated expression of the transgene. One example of such a system is the cre/loxP recombinase system of bacteriophage P1. For a description of the cre/loxP recombinase system, *See, e.g.*, Lakso, *et al.*, 1992. *Proc. Natl. Acad. Sci. USA* 89: 6232-6236. Another example of a recombinase system is the FLP recombinase system of *Saccharomyces cerevisiae. See,* O'Gorman, *et al.*, 1991. *Science* 251:1351-1355. If a cre/loxP recombinase system is used to regulate expression of the transgene, animals containing transgenes encoding both the Cre recombinase and a selected protein are required. Such animals can be provided through the construction of "double" transgenic animals, *e.g.*, by mating two transgenic animals, one containing a transgene encoding a selected protein and the other containing a transgene encoding a recombinase.

Clones of the non-human transgenic animals described herein can also be produced according to the methods described in Wilmut, *et al.*, 1997. *Nature* 385: 810-813. In brief, a cell (*e.g.*, a somatic cell) from the transgenic animal can be isolated and induced to exit the growth cycle and enter G_0 phase. The quiescent cell can then be fused, *e.g.*, through the use of electrical pulses, to an enucleated oocyte from an animal of the same species from which the quiescent cell is isolated. The reconstructed oocyte is then cultured such that it develops to morula or blastocyte and then transferred to pseudopregnant female foster animal. The offspring borne of this female foster animal will be a clone of the animal from which the cell (*e.g.*, the somatic cell) is isolated.

Pharmaceutical Compositions

The FCTRX nucleic acid molecules, FCTRX proteins, and anti-FCTRX antibodies (also referred to herein as "active compounds") of the invention, and derivatives, fragments, analogs and homologs thereof, can be incorporated into pharmaceutical compositions suitable for administration. Such compositions typically comprise the nucleic acid molecule, protein, or antibody and a pharmaceutically acceptable carrier. As used herein, "pharmaceutically acceptable carrier" is intended to include any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like,

compatible with pharmaceutical administration. Suitable carriers are described in the most recent edition of Remington's Pharmaceutical Sciences, a standard reference text in the field, which is incorporated herein by reference. Preferred examples of such carriers or diluents include, but are not limited to, water, saline, finger's solutions, dextrose solution, and 5% human serum albumin. Liposomes and non-aqueous vehicles such as fixed oils may also be used. The use of such media and agents for pharmaceutically active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the active compound, use thereof in the compositions is contemplated. Supplementary active compounds can also be incorporated into the compositions.

A pharmaceutical composition of the invention is formulated to be compatible with its intended route of administration. Examples of routes of administration include parenteral, *e.g.*, intravenous, intradermal, subcutaneous, oral (*e.g.*, inhalation), transdermal (*i.e.*, topical), transmucosal, and rectal administration. Solutions or suspensions used for parenteral, intradermal, or subcutaneous application can include the following components: a sterile diluent such as water for injection, saline solution, fixed oils, polyethylene glycols, glycerine, propylene glycol or other synthetic solvents; antibacterial agents such as benzyl alcohol or methyl parabens; antioxidants such as ascorbic acid or sodium bisulfite; chelating agents such as ethylenediaminetetraacetic acid (EDTA); buffers such as acetates, citrates or phosphates, and agents for the adjustment of tonicity such as sodium chloride or dextrose. The pH can be adjusted with acids or bases, such as hydrochloric acid or sodium hydroxide. The parenteral preparation can be enclosed in ampoules, disposable syringes or multiple dose vials made of glass or plastic.

Pharmaceutical compositions suitable for injectable use include sterile aqueous solutions (where water soluble) or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. For intravenous administration, suitable carriers include physiological saline, bacteriostatic water, Cremophor EL™ (BASF, Parsippany, N.J.) or phosphate buffered saline (PBS). In all cases, the composition must be sterile and should be fluid to the extent that easy syringeability exists. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), and suitable mixtures thereof. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. Prevention of the

action of microorganisms can be achieved by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, ascorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars, polyalcohols such as manitol, sorbitol, sodium chloride in the composition. Prolonged absorption of the injectable compositions can be brought about by including in the composition an agent which delays absorption, for example, aluminum monostearate and gelatin.

Sterile injectable solutions can be prepared by incorporating the active compound (*e.g.*, an FCTRX protein or anti-FCTRX antibody) in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the active compound into a sterile vehicle that contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, methods of preparation are vacuum drying and freeze-drying that yields a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

Oral compositions generally include an inert diluent or an edible carrier. They can be enclosed in gelatin capsules or compressed into tablets. For the purpose of oral therapeutic administration, the active compound can be incorporated with excipients and used in the form of tablets, troches, or capsules. Oral compositions can also be prepared using a fluid carrier for use as a mouthwash, wherein the compound in the fluid carrier is applied orally and swished and expectorated or swallowed. Pharmaceutically compatible binding agents, and/or adjuvant materials can be included as part of the composition. The tablets, pills, capsules, troches and the like can contain any of the following ingredients, or compounds of a similar nature: a binder such as microcrystalline cellulose, gum tragacanth or gelatin; an excipient such as starch or lactose, a disintegrating agent such as alginic acid, Primogel, or corn starch; a lubricant such as magnesium stearate or Sterotes; a glidant such as colloidal silicon dioxide; a sweetening agent such as sucrose or saccharin; or a flavoring agent such as peppermint, methyl salicylate, or orange flavoring.

For administration by inhalation, the compounds are delivered in the form of an aerosol spray from pressured container or dispenser which contains a suitable propellant, *e.g.*, a gas such as carbon dioxide, or a nebulizer.

Systemic administration can also be by transmucosal or transdermal means. For transmucosal or transdermal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art, and include, for

example, for transmucosal administration, detergents, bile salts, and fusidic acid derivatives. Transmucosal administration can be accomplished through the use of nasal sprays or suppositories. For transdermal administration, the active compounds are formulated into ointments, salves, gels, or creams as generally known in the art.

The compounds can also be prepared in the form of suppositories (*e.g.*, with conventional suppository bases such as cocoa butter and other glycerides) or retention enemas for rectal delivery.

In one embodiment, the active compounds are prepared with carriers that will protect the compound against rapid elimination from the body, such as a controlled release formulation, including implants and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Methods for preparation of such formulations will be apparent to those skilled in the art. The materials can also be obtained commercially from Alza Corporation and Nova Pharmaceuticals, Inc. Liposomal suspensions (including liposomes targeted to infected cells with monoclonal antibodies to viral antigens) can also be used as pharmaceutically acceptable carriers. These can be prepared according to methods known to those skilled in the art, for example, as described in U.S. Patent No. 4,522,811.

It is especially advantageous to formulate oral or parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the subject to be treated; each unit containing a predetermined quantity of active compound calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. The specification for the dosage unit forms of the invention are dictated by and directly dependent on the unique characteristics of the active compound and the particular therapeutic effect to be achieved, and the limitations inherent in the art of compounding such an active compound for the treatment of individuals.

The nucleic acid molecules of the invention can be inserted into vectors and used as gene therapy vectors. Gene therapy vectors can be delivered to a subject by, for example, intravenous injection, local administration (*see, e.g.,* U.S. Patent No. 5,328,470) or by stereotactic injection (*see, e.g.*, Chen, *et al.*, 1994. *Proc. Natl. Acad. Sci. USA* 91: 3054-3057). The pharmaceutical preparation of the gene therapy vector can include the gene therapy vector in an acceptable diluent, or can comprise a slow release matrix in which the gene delivery vehicle is imbedded. Alternatively, where the complete gene delivery vector can be produced intact from recombinant

cells, *e.g.*, retroviral vectors, the pharmaceutical preparation can include one or more cells that produce the gene delivery system.

The pharmaceutical compositions can be included in a container, pack, or dispenser together with instructions for administration.

Screening and Detection Methods

The isolated nucleic acid molecules of the invention can be used to express FCTRX protein (*e.g.*, via a recombinant expression vector in a host cell in gene therapy applications), to detect FCTRX mRNA (*e.g.*, in a biological sample) or a genetic lesion in an FCTRX gene, and to modulate FCTRX activity, as described further, below. In addition, the FCTRX proteins can be used to screen drugs or compounds that modulate the FCTRX protein activity or expression as well as to treat disorders characterized by insufficient or excessive production of FCTRX protein or production of FCTRX protein forms that have decreased or aberrant activity compared to FCTRX wild-type protein (*e.g.*; diabetes (regulates insulin release); obesity (binds and transport lipids); metabolic disturbances associated with obesity, the metabolic syndrome X as well as anorexia and wasting disorders associated with chronic diseases and various cancers, and infectious disease(possesses anti-microbial activity) and the various dyslipidemias. In addition, the anti-FCTRX antibodies of the invention can be used to detect and isolate FCTRX proteins and modulate FCTRX activity. In yet a further aspect, the invention can be used in methods to influence appetite, absorption of nutrients and the disposition of metabolic substrates in both a positive and negative fashion.

The invention further pertains to novel agents identified by the screening assays described herein and uses thereof for treatments as described, *supra*.

Screening Assays

The invention provides a method (also referred to herein as a "screening assay") for identifying modulators, *i.e.*, candidate or test compounds or agents (*e.g.*, peptides, peptidomimetics, small molecules or other drugs) that bind to FCTRX proteins or have a stimulatory or inhibitory effect on, *e.g.*, FCTRX protein expression or FCTRX protein activity. The invention also includes compounds identified in the screening assays described herein.

In one embodiment, the invention provides assays for screening candidate or test compounds which bind to or modulate the activity of the membrane-bound form of an FCTRX protein or polypeptide or biologically-active portion thereof. The test compounds of the invention can be obtained using any of the numerous approaches in combinatorial library methods known in the art, including: biological libraries; spatially addressable parallel solid

phase or solution phase libraries; synthetic library methods requiring deconvolution; the "one-bead one-compound" library method; and synthetic library methods using affinity chromatography selection. The biological library approach is limited to peptide libraries, while the other four approaches are applicable to peptide, non-peptide oligomer or small molecule libraries of compounds. *See, e.g.,* Lam, 1997. *Anticancer Drug Design* 12: 145.

A "small molecule" as used herein, is meant to refer to a composition that has a molecular weight of less than about 5 kD and most preferably less than about 4 kD. Small molecules can be, *e.g.*, nucleic acids, peptides, polypeptides, peptidomimetics, carbohydrates, lipids or other organic or inorganic molecules. Libraries of chemical and/or biological mixtures, such as fungal, bacterial, or algal extracts, are known in the art and can be screened with any of the assays of the invention.

Examples of methods for the synthesis of molecular libraries can be found in the art, for example in: DeWitt, *et al.*, 1993. *Proc. Natl. Acad. Sci. U.S.A.* 90: 6909; Erb, *et al.*, 1994. *Proc. Natl. Acad. Sci. U.S.A.* 91: 11422; Zuckermann, *et al.*, 1994. *J. Med. Chem.* 37: 2678; Cho, *et al.*, 1993. *Science* 261: 1303; Carrell, *et al.*, 1994. *Angew. Chem. Int. Ed. Engl.* 33: 2059; Carell, *et al.*, 1994. *Angew. Chem. Int. Ed. Engl.* 33: 2061; and Gallop, *et al.*, 1994. *J. Med. Chem.* 37: 1233.

Libraries of compounds may be presented in solution (*e.g.*, Houghten, 1992. *Biotechniques* 13: 412-421), or on beads (Lam, 1991. *Nature* 354: 82-84), on chips (Fodor, 1993. *Nature* 364: 555-556), bacteria (Ladner, U.S. Patent No. 5,223,409), spores (Ladner, U.S. Patent 5,233,409), plasmids (Cull, *et al.*, 1992. *Proc. Natl. Acad. Sci. USA* 89: 1865-1869) or on phage (Scott and Smith, 1990. *Science* 249: 386-390; Devlin, 1990. *Science* 249: 404-406; Cwirla, *et al.*, 1990. *Proc. Natl. Acad. Sci. U.S.A.* 87: 6378-6382; Felici, 1991. *J. Mol. Biol.* 222: 301-310; Ladner, U.S. Patent No. 5,233,409.).

In one embodiment, an assay is a cell-based assay in which a cell which expresses a membrane-bound form of FCTRX protein, or a biologically-active portion thereof, on the cell surface is contacted with a test compound and the ability of the test compound to bind to an FCTRX protein determined. The cell, for example, can of mammalian origin or a yeast cell. Determining the ability of the test compound to bind to the FCTRX protein can be accomplished, for example, by coupling the test compound with a radioisotope or enzymatic label such that binding of the test compound to the FCTRX protein or biologically-active portion thereof can be determined by detecting the labeled compound in a complex. For example, test compounds can be labeled with ^{125}I, ^{35}S, ^{14}C, or ^{3}H, either directly or indirectly, and the radioisotope detected by direct counting of radioemission or by scintillation counting. Alternatively, test compounds can

be enzymatically-labeled with, for example, horseradish peroxidase, alkaline phosphatase, or luciferase, and the enzymatic label detected by determination of conversion of an appropriate substrate to product. In one embodiment, the assay comprises contacting a cell which expresses a membrane-bound form of FCTRX protein, or a biologically-active portion thereof, on the cell surface with a known compound which binds FCTRX to form an assay mixture, contacting the assay mixture with a test compound, and determining the ability of the test compound to interact with an FCTRX protein, wherein determining the ability of the test compound to interact with an FCTRX protein comprises determining the ability of the test compound to preferentially bind to FCTRX protein or a biologically-active portion thereof as compared to the known compound.

In another embodiment, an assay is a cell-based assay comprising contacting a cell expressing a membrane-bound form of FCTRX protein, or a biologically-active portion thereof, on the cell surface with a test compound and determining the ability of the test compound to modulate (*e.g.*, stimulate or inhibit) the activity of the FCTRX protein or biologically-active portion thereof. Determining the ability of the test compound to modulate the activity of FCTRX or a biologically-active portion thereof can be accomplished, for example, by determining the ability of the FCTRX protein to bind to or interact with an FCTRX target molecule. As used herein, a "target molecule" is a molecule with which an FCTRX protein binds or interacts in nature, for example, a molecule on the surface of a cell which expresses an FCTRX interacting protein, a molecule on the surface of a second cell, a molecule in the extracellular milieu, a molecule associated with the internal surface of a cell membrane or a cytoplasmic molecule. An FCTRX target molecule can be a non-FCTRX molecule or an FCTRX protein or polypeptide of the invention. In one embodiment, an FCTRX target molecule is a component of a signal transduction pathway that facilitates transduction of an extracellular signal (*e.g.* a signal generated by binding of a compound to a membrane-bound FCTRX molecule) through the cell membrane and into the cell. The target, for example, can be a second intercellular protein that has catalytic activity or a protein that facilitates the association of downstream signaling molecules with FCTRX.

Determining the ability of the FCTRX protein to bind to or interact with an FCTRX target molecule can be accomplished by one of the methods described above for determining direct binding. In one embodiment, determining the ability of the FCTRX protein to bind to or interact with an FCTRX target molecule can be accomplished by determining the activity of the target molecule. For example, the activity of the target molecule can be determined by detecting induction of a cellular second messenger of the target (*i.e.* intracellular Ca^{2+}, diacylglycerol, IP_3, etc.), detecting catalytic/enzymatic activity of the target an appropriate substrate, detecting the

induction of a reporter gene (comprising an FCTRX-responsive regulatory element operatively linked to a nucleic acid encoding a detectable marker, *e.g.*, luciferase), or detecting a cellular response, for example, cell survival, cellular differentiation, or cell proliferation.

In yet another embodiment, an assay of the invention is a cell-free assay comprising contacting an FCTRX protein or biologically-active portion thereof with a test compound and determining the ability of the test compound to bind to the FCTRX protein or biologically-active portion thereof. Binding of the test compound to the FCTRX protein can be determined either directly or indirectly as described above. In one such embodiment, the assay comprises contacting the FCTRX protein or biologically-active portion thereof with a known compound which binds FCTRX to form an assay mixture, contacting the assay mixture with a test compound, and determining the ability of the test compound to interact with an FCTRX protein, wherein determining the ability of the test compound to interact with an FCTRX protein comprises determining the ability of the test compound to preferentially bind to FCTRX or biologically-active portion thereof as compared to the known compound.

In still another embodiment, an assay is a cell-free assay comprising contacting FCTRX protein or biologically-active portion thereof with a test compound and determining the ability of the test compound to modulate (*e.g.* stimulate or inhibit) the activity of the FCTRX protein or biologically-active portion thereof. Determining the ability of the test compound to modulate the activity of FCTRX can be accomplished, for example, by determining the ability of the FCTRX protein to bind to an FCTRX target molecule by one of the methods described above for determining direct binding. In an alternative embodiment, determining the ability of the test compound to modulate the activity of FCTRX protein can be accomplished by determining the ability of the FCTRX protein further modulate an FCTRX target molecule. For example, the catalytic/enzymatic activity of the target molecule on an appropriate substrate can be determined as described, *supra*.

In yet another embodiment, the cell-free assay comprises contacting the FCTRX protein or biologically-active portion thereof with a known compound which binds FCTRX protein to form an assay mixture, contacting the assay mixture with a test compound, and determining the ability of the test compound to interact with an FCTRX protein, wherein determining the ability of the test compound to interact with an FCTRX protein comprises determining the ability of the FCTRX protein to preferentially bind to or modulate the activity of an FCTRX target molecule.

The cell-free assays of the invention are amenable to use of both the soluble form or the membrane-bound form of FCTRX protein. In the case of cell-free assays comprising the membrane-bound form of FCTRX protein, it may be desirable to utilize a solubilizing agent such

that the membrane-bound form of FCTRX protein is maintained in solution. Examples of such solubilizing agents include non-ionic detergents such as n-octylglucoside, n-dodecylglucoside, n-dodecylmaltoside, octanoyl-N-methylglucamide, decanoyl-N-methylglucamide, Triton® X-100, Triton® X-114, Thesit®, Isotridecypoly(ethylene glycol ether)$_n$, N-dodecyl--N,N-dimethyl-3-ammonio-1-propane sulfonate, 3-(3-cholamidopropyl) dimethylamminiol-1-propane sulfonate (CHAPS), or 3-(3-cholamidopropyl)dimethylamminiol-2-hydroxy-1-propane sulfonate (CHAPSO).

In more than one embodiment of the above assay methods of the invention, it may be desirable to immobilize either FCTRX protein or its target molecule to facilitate separation of complexed from uncomplexed forms of one or both of the proteins, as well as to accommodate automation of the assay. Binding of a test compound to FCTRX protein, or interaction of FCTRX protein with a target molecule in the presence and absence of a candidate compound, can be accomplished in any vessel suitable for containing the reactants. Examples of such vessels include microtiter plates, test tubes, and micro-centrifuge tubes. In one embodiment, a fusion protein can be provided that adds a domain that allows one or both of the proteins to be bound to a matrix. For example, GST-FCTRX fusion proteins or GST-target fusion proteins can be adsorbed onto glutathione sepharose beads (Sigma Chemical, St. Louis, MO) or glutathione derivatized microtiter plates, that are then combined with the test compound or the test compound and either the non-adsorbed target protein or FCTRX protein, and the mixture is incubated under conditions conducive to complex formation (*e.g.*, at physiological conditions for salt and pH). Following incubation, the beads or microtiter plate wells are washed to remove any unbound components, the matrix immobilized in the case of beads, complex determined either directly or indirectly, for example, as described, *supra*. Alternatively, the complexes can be dissociated from the matrix, and the level of FCTRX protein binding or activity determined using standard techniques.

Other techniques for immobilizing proteins on matrices can also be used in the screening assays of the invention. For example, either the FCTRX protein or its target molecule can be immobilized utilizing conjugation of biotin and streptavidin. Biotinylated FCTRX protein or target molecules can be prepared from biotin-NHS (N-hydroxy-succinimide) using techniques well-known within the art (*e.g.*, biotinylation kit, Pierce Chemicals, Rockford, Ill.), and immobilized in the wells of streptavidin-coated 96 well plates (Pierce Chemical). Alternatively, antibodies reactive with FCTRX protein or target molecules, but which do not interfere with binding of the FCTRX protein to its target molecule, can be derivatized to the wells of the plate, and unbound target or FCTRX protein trapped in the wells by antibody conjugation. Methods

for detecting such complexes, in addition to those described above for the GST-immobilized complexes, include immunodetection of complexes using antibodies reactive with the FCTRX protein or target molecule, as well as enzyme-linked assays that rely on detecting an enzymatic activity associated with the FCTRX protein or target molecule.

In another embodiment, modulators of FCTRX protein expression are identified in a method wherein a cell is contacted with a candidate compound and the expression of FCTRX mRNA or protein in the cell is determined. The level of expression of FCTRX mRNA or protein in the presence of the candidate compound is compared to the level of expression of FCTRX mRNA or protein in the absence of the candidate compound. The candidate compound can then be identified as a modulator of FCTRX mRNA or protein expression based upon this comparison. For example, when expression of FCTRX mRNA or protein is greater (*i.e.*, statistically significantly greater) in the presence of the candidate compound than in its absence, the candidate compound is identified as a stimulator of FCTRX mRNA or protein expression. Alternatively, when expression of FCTRX mRNA or protein is less (statistically significantly less) in the presence of the candidate compound than in its absence, the candidate compound is identified as an inhibitor of FCTRX mRNA or protein expression. The level of FCTRX mRNA or protein expression in the cells can be determined by methods described herein for detecting FCTRX mRNA or protein.

In yet another aspect of the invention, the FCTRX proteins can be used as "bait proteins" in a two-hybrid assay or three hybrid assay (*see, e.g.*, U.S. Patent No. 5,283,317; Zervos, *et al.*, 1993. *Cell* 72: 223-232; Madura, *et al.*, 1993. *J. Biol. Chem.* 268: 12046-12054; Bartel, *et al.*, 1993. *Biotechniques* 14: 920-924; Iwabuchi, *et al.*, 1993. *Oncogene* 8: 1693-1696; and Brent WO 94/10300), to identify other proteins that bind to or interact with FCTRX ("FCTRX-binding proteins" or "FCTRX-bp") and modulate FCTRX activity. Such FCTRX-binding proteins are also likely to be involved in the propagation of signals by the FCTRX proteins as, for example, upstream or downstream elements of the FCTRX pathway.

The two-hybrid system is based on the modular nature of most transcription factors, which consist of separable DNA-binding and activation domains. Briefly, the assay utilizes two different DNA constructs. In one construct, the gene that codes for FCTRX is fused to a gene encoding the DNA binding domain of a known transcription factor (*e.g.*, GAL-4). In the other construct, a DNA sequence, from a library of DNA sequences, that encodes an unidentified protein ("prey" or "sample") is fused to a gene that codes for the activation domain of the known transcription factor. If the "bait" and the "prey" proteins are able to interact, *in vivo*, forming an FCTRX-dependent complex, the DNA-binding and activation domains of the transcription factor

are brought into close proximity. This proximity allows transcription of a reporter gene (*e.g.*, LacZ) that is operably linked to a transcriptional regulatory site responsive to the transcription factor. Expression of the reporter gene can be detected and cell colonies containing the functional transcription factor can be isolated and used to obtain the cloned gene that encodes the protein which interacts with FCTRX.

The invention further pertains to novel agents identified by the aforementioned screening assays and uses thereof for treatments as described herein.

Detection Assays

Portions or fragments of the cDNA sequences identified herein (and the corresponding complete gene sequences) can be used in numerous ways as polynucleotide reagents. By way of example, and not of limitation, these sequences can be used to: (*i*) map their respective genes on a chromosome; and, thus, locate gene regions associated with genetic disease; (*ii*) identify an individual from a minute biological sample (tissue typing); and (*iii*) aid in forensic identification of a biological sample. Some of these applications are described in the subsections, below.

Chromosome Mapping

Once the sequence (or a portion of the sequence) of a gene has been isolated, this sequence can be used to map the location of the gene on a chromosome. This process is called chromosome mapping. Accordingly, portions or fragments of the FCTRX sequences, SEQ ID NOS:1, 3, 5, 7, 9, 10, 11, 12, 14, 16, 18, 20, 22, and 24, or fragments or derivatives thereof, can be used to map the location of the FCTRX genes, respectively, on a chromosome. The mapping of the FCTRX sequences to chromosomes is an important first step in correlating these sequences with genes associated with disease.

Briefly, FCTRX genes can be mapped to chromosomes by preparing PCR primers (preferably 15-25 bp in length) from the FCTRX sequences. Computer analysis of the FCTRX, sequences can be used to rapidly select primers that do not span more than one exon in the genomic DNA, thus complicating the amplification process. These primers can then be used for PCR screening of somatic cell hybrids containing individual human chromosomes. Only those hybrids containing the human gene corresponding to the FCTRX sequences will yield an amplified fragment.

Somatic cell hybrids are prepared by fusing somatic cells from different mammals (*e.g.*, human and mouse cells). As hybrids of human and mouse cells grow and divide, they gradually lose human chromosomes in random order, but retain the mouse chromosomes. By using media in which mouse cells cannot grow, because they lack a particular enzyme, but in which human

cells can, the one human chromosome that contains the gene encoding the needed enzyme will be retained. By using various media, panels of hybrid cell lines can be established. Each cell line in a panel contains either a single human chromosome or a small number of human chromosomes, and a full set of mouse chromosomes, allowing easy mapping of individual genes to specific human chromosomes. *See, e.g.,* D'Eustachio, *et al.*, 1983. *Science* 220: 919-924. Somatic cell hybrids containing only fragments of human chromosomes can also be produced by using human chromosomes with translocations and deletions.

PCR mapping of somatic cell hybrids is a rapid procedure for assigning a particular sequence to a particular chromosome. Three or more sequences can be assigned per day using a single thermal cycler. Using the FCTRX sequences to design oligonucleotide primers, sub-localization can be achieved with panels of fragments from specific chromosomes.

Fluorescence *in situ* hybridization (FISH) of a DNA sequence to a metaphase chromosomal spread can further be used to provide a precise chromosomal location in one step. Chromosome spreads can be made using cells whose division has been blocked in metaphase by a chemical like colcemid that disrupts the mitotic spindle. The chromosomes can be treated briefly with trypsin, and then stained with Giemsa. A pattern of light and dark bands develops on each chromosome, so that the chromosomes can be identified individually. The FISH technique can be used with a DNA sequence as short as 500 or 600 bases. However, clones larger than 1,000 bases have a higher likelihood of binding to a unique chromosomal location with sufficient signal intensity for simple detection. Preferably 1,000 bases, and more preferably 2,000 bases, will suffice to get good results at a reasonable amount of time. For a review of this technique, *see,* Verma, *et al.*, HUMAN CHROMOSOMES: A MANUAL OF BASIC TECHNIQUES (Pergamon Press, New York 1988).

Reagents for chromosome mapping can be used individually to mark a single chromosome or a single site on that chromosome, or panels of reagents can be used for marking multiple sites and/or multiple chromosomes. Reagents corresponding to noncoding regions of the genes actually are preferred for mapping purposes. Coding sequences are more likely to be conserved within gene families, thus increasing the chance of cross hybridizations during chromosomal mapping.

Once a sequence has been mapped to a precise chromosomal location, the physical position of the sequence on the chromosome can be correlated with genetic map data. Such data are found, *e.g.,* in McKusick, MENDELIAN INHERITANCE IN MAN, available on-line through Johns Hopkins University Welch Medical Library). The relationship between genes and disease, mapped to the same chromosomal region, can then be identified through linkage analysis

(co-inheritance of physically adjacent genes), described in, *e.g.*, Egeland, *et al.*, 1987. *Nature*, 325: 783-787.

Moreover, differences in the DNA sequences between individuals affected and unaffected with a disease associated with the FCTRX gene, can be determined. If a mutation is observed in some or all of the affected individuals but not in any unaffected individuals, then the mutation is likely to be the causative agent of the particular disease. Comparison of affected and unaffected individuals generally involves first looking for structural alterations in the chromosomes, such as deletions or translocations that are visible from chromosome spreads or detectable using PCR based on that DNA sequence. Ultimately, complete sequencing of genes from several individuals can be performed to confirm the presence of a mutation and to distinguish mutations from polymorphisms.

Tissue Typing

The FCTRX sequences of the invention can also be used to identify individuals from minute biological samples. In this technique, an individual's genomic DNA is digested with one or more restriction enzymes, and probed on a Southern blot to yield unique bands for identification. The sequences of the invention are useful as additional DNA markers for RFLP ("restriction fragment length polymorphisms," described in U.S. Patent No. 5,272,057).

Furthermore, the sequences of the invention can be used to provide an alternative technique that determines the actual base-by-base DNA sequence of selected portions of an individual's genome. Thus, the FCTRX sequences described herein can be used to prepare two PCR primers from the 5'- and 3'-termini of the sequences. These primers can then be used to amplify an individual's DNA and subsequently sequence it.

Panels of corresponding DNA sequences from individuals, prepared in this manner, can provide unique individual identifications, as each individual will have a unique set of such DNA sequences due to allelic differences. The sequences of the invention can be used to obtain such identification sequences from individuals and from tissue. The FCTRX sequences of the invention uniquely represent portions of the human genome. Allelic variation occurs to some degree in the coding regions of these sequences, and to a greater degree in the noncoding regions. It is estimated that allelic variation between individual humans occurs with a frequency of about once per each 500 bases. Much of the allelic variation is due to single nucleotide polymorphisms (SNPs), which include restriction fragment length polymorphisms (RFLPs).

Each of the sequences described herein can, to some degree, be used as a standard against which DNA from an individual can be compared for identification purposes. Because greater numbers of polymorphisms occur in the noncoding regions, fewer sequences are necessary to

differentiate individuals. The noncoding sequences can comfortably provide positive individual identification with a panel of perhaps 10 to 1,000 primers that each yield a noncoding amplified sequence of 100 bases. If predicted coding sequences, such as those in SEQ ID NOS:1, 3, 5, 7, 9, 10, 11, 12, 14, 16, 18, 20, 22, and 24, are used, a more appropriate number of primers for positive individual identification would be 500-2,000.

Predictive Medicine

The invention also pertains to the field of predictive medicine in which diagnostic assays, prognostic assays, pharmacogenomics, and monitoring clinical trials are used for prognostic (predictive) purposes to thereby treat an individual prophylactically. Accordingly, one aspect of the invention relates to diagnostic assays for determining FCTRX protein and/or nucleic acid expression as well as FCTRX activity, in the context of a biological sample (*e.g.*, blood, serum, cells, tissue) to thereby determine whether an individual is afflicted with a disease or disorder, or is at risk of developing a disorder, associated with aberrant FCTRX expression or activity. The disorders include Also within the scope of the invention is the use of a Therapeutic in the manufacture of a medicament for treating or preventing disorders or syndromes including, *e.g.*, Colorectal cancer, adenomatous polyposis coli, myelogenous leukemia, congenital ceonatal alloimmune thrombocytopenia, multiple human solid malignancies, malignant ovarian tumours particularly at the interface between epithelia and stroma, malignant brain tumors, mammary tumors, human gliomas, astrocytomas, mixed glioma/astrocytomas, renal cells carcinoma, breast adenocarcinoma, ovarian cancer, melanomas, renal cell carcinoma , clear cell and granular cell carcinomas, autocrine/paracrine stimulation of tumor cell proliferation, autocrine/paracrine stimulation of tumor cell survival and tumor cell resistance to cytotoxic therapy, paranechmal and basement membrane invasion and motility of tumor cells thereby contributing to metastasis, tumor-mediated immunosuppression of T-cell mediated immune effector cells and pathways resulting in tumor escape from immune surveilance, neurological disorders, neurodegenerative disorders, nerve trauma, familial myelodysplastic syndrome, Charcot-Marie-Tooth neuropathy, demyelinating Gardner syndrome, familial myelodysplastic syndrome; mental health conditions, immunological disorders, allergy and infection, asthma, bronchial asthma, Avellino type eosinophilia, lung diseases, reproductive disorders, male infertility, female reproductive system disorders, male and female reproductive diseases, hemangioma, deafness, glycoprotein Ia deficiency, desmoid disease, turcot syndrome, liver cirrhosis, hepatitis C, gastric disorders, pancreatic diseases like diabetes, Schistosoma mansoni infection, Spinocerebellar ataxia, Plasmodium falciparum parasitemia, Corneal dystrophy -Groenouw type I, Corneal dystrophy - lattice type I, and Reis-Bucklers corneal dystrophy. The invention also provides for prognostic

(or predictive) assays for determining whether an individual is at risk of developing a disorder associated with FCTRX protein, nucleic acid expression or activity. For example, mutations in an FCTRX gene can be assayed in a biological sample. Such assays can be used for prognostic or predictive purpose to thereby prophylactically treat an individual prior to the onset of a disorder characterized by or associated with FCTRX protein, nucleic acid expression, or biological activity.

Another aspect of the invention provides methods for determining FCTRX protein, nucleic acid expression or activity in an individual to thereby select appropriate therapeutic or prophylactic agents for that individual (referred to herein as "pharmacogenomics"). Pharmacogenomics allows for the selection of agents (*e.g.*, drugs) for therapeutic or prophylactic treatment of an individual based on the genotype of the individual (*e.g.*, the genotype of the individual examined to determine the ability of the individual to respond to a particular agent.)

Yet another aspect of the invention pertains to monitoring the influence of agents (*e.g.*, drugs, compounds) on the expression or activity of FCTRX in clinical trials.

These and other agents are described in further detail in the following sections.

Diagnostic Assays

An exemplary method for detecting the presence or absence of FCTRX in a biological sample involves obtaining a biological sample from a test subject and contacting the biological sample with a compound or an agent capable of detecting FCTRX protein or nucleic acid (*e.g.*, mRNA, genomic DNA) that encodes FCTRX protein such that the presence of FCTRX is detected in the biological sample. An agent for detecting FCTRX mRNA or genomic DNA is a labeled nucleic acid probe capable of hybridizing to FCTRX mRNA or genomic DNA. The nucleic acid probe can be, for example, a full-length FCTRX nucleic acid, such as the nucleic acid of SEQ ID NOS:1, 3, 5, 7, 9, 10, 11, 12, 14, 16, 18, 20, 22, and 24, or a portion thereof, such as an oligonucleotide of at least 15, 30, 50, 100, 250 or 500 nucleotides in length and sufficient to specifically hybridize under stringent conditions to FCTRX mRNA or genomic DNA. Other suitable probes for use in the diagnostic assays of the invention are described herein.

An agent for detecting FCTRX protein is an antibody capable of binding to FCTRX protein, preferably an antibody with a detectable label. Antibodies can be polyclonal, or more preferably, monoclonal. An intact antibody, or a fragment thereof (*e.g.*, Fab or $F(ab')_2$) can be used. The term "labeled", with regard to the probe or antibody, is intended to encompass direct labeling of the probe or antibody by coupling (*i.e.*, physically linking) a detectable substance to the probe or antibody, as well as indirect labeling of the probe or antibody by reactivity with another reagent that is directly labeled. Examples of indirect labeling include detection of a

primary antibody using a fluorescently-labeled secondary antibody and end-labeling of a DNA probe with biotin such that it can be detected with fluorescently-labeled streptavidin. The term "biological sample" is intended to include tissues, cells and biological fluids isolated from a subject, as well as tissues, cells and fluids present within a subject. That is, the detection method of the invention can be used to detect FCTRX mRNA, protein, or genomic DNA in a biological sample *in vitro* as well as *in vivo*. For example, *in vitro* techniques for detection of FCTRX mRNA include Northern hybridizations and *in situ* hybridizations. *In vitro* techniques for detection of FCTRX protein include enzyme linked immunosorbent assays (ELISAs), Western blots, immunoprecipitations, and immunofluorescence. *In vitro* techniques for detection of FCTRX genomic DNA include Southern hybridizations. Furthermore, *in vivo* techniques for detection of FCTRX protein include introducing into a subject a labeled anti-FCTRX antibody. For example, the antibody can be labeled with a radioactive marker whose presence and location in a subject can be detected by standard imaging techniques.

In one embodiment, the biological sample contains protein molecules from the test subject. Alternatively, the biological sample can contain mRNA molecules from the test subject or genomic DNA molecules from the test subject. A preferred biological sample is a peripheral blood leukocyte sample isolated by conventional means from a subject.

In another embodiment, the methods further involve obtaining a control biological sample from a control subject, contacting the control sample with a compound or agent capable of detecting FCTRX protein, mRNA, or genomic DNA, such that the presence of FCTRX protein, mRNA or genomic DNA is detected in the biological sample, and comparing the presence of FCTRX protein, mRNA or genomic DNA in the control sample with the presence of FCTRX protein, mRNA or genomic DNA in the test sample.

The invention also encompasses kits for detecting the presence of FCTRX in a biological sample. For example, the kit can comprise: a labeled compound or agent capable of detecting FCTRX protein or mRNA in a biological sample; means for determining the amount of FCTRX in the sample; and means for comparing the amount of FCTRX in the sample with a standard. The compound or agent can be packaged in a suitable container. The kit can further comprise instructions for using the kit to detect FCTRX protein or nucleic acid.

Prognostic Assays

The diagnostic methods described herein can furthermore be utilized to identify subjects having or at risk of developing a disease or disorder associated with aberrant FCTRX expression or activity. For example, the assays described herein, such as the preceding diagnostic assays or the following assays, can be utilized to identify a subject having or at risk of developing a

disorder associated with FCTRX protein, nucleic acid expression or activity. Alternatively, the prognostic assays can be utilized to identify a subject having or at risk for developing a disease or disorder. Thus, the invention provides a method for identifying a disease or disorder associated with aberrant FCTRX expression or activity in which a test sample is obtained from a subject and FCTRX protein or nucleic acid (*e.g.*, mRNA, genomic DNA) is detected, wherein the presence of FCTRX protein or nucleic acid is diagnostic for a subject having or at risk of developing a disease or disorder associated with aberrant FCTRX expression or activity. As used herein, a "test sample" refers to a biological sample obtained from a subject of interest. For example, a test sample can be a biological fluid (*e.g.*, serum), cell sample, or tissue.

Furthermore, the prognostic assays described herein can be used to determine whether a subject can be administered an agent (*e.g.*, an agonist, antagonist, peptidomimetic, protein, peptide, nucleic acid, small molecule, or other drug candidate) to treat a disease or disorder associated with aberrant FCTRX expression or activity. For example, such methods can be used to determine whether a subject can be effectively treated with an agent for a disorder. Thus, the invention provides methods for determining whether a subject can be effectively treated with an agent for a disorder associated with aberrant FCTRX expression or activity in which a test sample is obtained and FCTRX protein or nucleic acid is detected (*e.g.*, wherein the presence of FCTRX protein or nucleic acid is diagnostic for a subject that can be administered the agent to treat a disorder associated with aberrant FCTRX expression or activity).

The methods of the invention can also be used to detect genetic lesions in an FCTRX gene, thereby determining if a subject with the lesioned gene is at risk for a disorder characterized by aberrant cell proliferation and/or differentiation. In various embodiments, the methods include detecting, in a sample of cells from the subject, the presence or absence of a genetic lesion characterized by at least one of an alteration affecting the integrity of a gene encoding an FCTRX-protein, or the misexpression of the FCTRX gene. For example, such genetic lesions can be detected by ascertaining the existence of at least one of: (*i*) a deletion of one or more nucleotides from an FCTRX gene; (*ii*) an addition of one or more nucleotides to an FCTRX gene; (*iii*) a substitution of one or more nucleotides of an FCTRX gene, (*iv*) a chromosomal rearrangement of an FCTRX gene; (*v*) an alteration in the level of a messenger RNA transcript of an FCTRX gene, (*vi*) aberrant modification of an FCTRX gene, such as of the methylation pattern of the genomic DNA, (*vii*) the presence of a non-wild-type splicing pattern of a messenger RNA transcript of an FCTRX gene, (*viii*) a non-wild-type level of an FCTRX protein, (*ix*) allelic loss of an FCTRX gene, and (*x*) inappropriate post-translational modification of an FCTRX protein. As described herein, there are a large number of assay techniques known

in the art which can be used for detecting lesions in an FCTRX gene. A preferred biological sample is a peripheral blood leukocyte sample isolated by conventional means from a subject. However, any biological sample containing nucleated cells may be used, including, for example, buccal mucosal cells.

In certain embodiments, detection of the lesion involves the use of a probe/primer in a polymerase chain reaction (PCR) (*see, e.g.*, U.S. Patent Nos. 4,683,195 and 4,683,202), such as anchor PCR or RACE PCR, or, alternatively, in a ligation chain reaction (LCR) (*see, e.g.*, Landegran, *et al.*, 1988. *Science* 241: 1077-1080; and Nakazawa, *et al.*, 1994. *Proc. Natl. Acad. Sci. USA* 91: 360-364), the latter of which can be particularly useful for detecting point mutations in the FCTRX-gene (*see,* Abravaya, *et al.*, 1995. *Nucl. Acids Res.* 23: 675-682). This method can include the steps of collecting a sample of cells from a patient, isolating nucleic acid (*e.g.*, genomic, mRNA or both) from the cells of the sample, contacting the nucleic acid sample with one or more primers that specifically hybridize to an FCTRX gene under conditions such that hybridization and amplification of the FCTRX gene (if present) occurs, and detecting the presence or absence of an amplification product, or detecting the size of the amplification product and comparing the length to a control sample. It is anticipated that PCR and/or LCR may be desirable to use as a preliminary amplification step in conjunction with any of the techniques used for detecting mutations described herein.

Alternative amplification methods include: self sustained sequence replication (*see,* Guatelli, *et al.*, 1990. *Proc. Natl. Acad. Sci. USA* 87: 1874-1878), transcriptional amplification system (*see,* Kwoh, *et al.*, 1989. *Proc. Natl. Acad. Sci. USA* 86: 1173-1177); Qβ Replicase (*see,* Lizardi, *et al*, 1988. *BioTechnology* 6: 1197), or any other nucleic acid amplification method, followed by the detection of the amplified molecules using techniques well known to those of skill in the art. These detection schemes are especially useful for the detection of nucleic acid molecules if such molecules are present in very low numbers.

In an alternative embodiment, mutations in an FCTRX gene from a sample cell can be identified by alterations in restriction enzyme cleavage patterns. For example, sample and control DNA is isolated, amplified (optionally), digested with one or more restriction endonucleases, and fragment length sizes are determined by gel electrophoresis and compared. Differences in fragment length sizes between sample and control DNA indicates mutations in the sample DNA. Moreover, the use of sequence specific ribozymes (*see, e.g.,* U.S. Patent No. 5,493,531) can be used to score for the presence of specific mutations by development or loss of a ribozyme cleavage site.

In other embodiments, genetic mutations in FCTRX can be identified by hybridizing a sample and control nucleic acids, *e.g.*, DNA or RNA, to high-density arrays containing hundreds or thousands of oligonucleotides probes. *See, e.g.,* Cronin, *et al.*, 1996. *Human Mutation* 7: 244-255; Kozal, *et al.*, 1996. *Nat. Med.* 2: 753-759. For example, genetic mutations in FCTRX can be identified in two dimensional arrays containing light-generated DNA probes as described in Cronin, *et al.*, *supra*. Briefly, a first hybridization array of probes can be used to scan through long stretches of DNA in a sample and control to identify base changes between the sequences by making linear arrays of sequential overlapping probes. This step allows the identification of point mutations. This is followed by a second hybridization array that allows the characterization of specific mutations by using smaller, specialized probe arrays complementary to all variants or mutations detected. Each mutation array is composed of parallel probe sets, one complementary to the wild-type gene and the other complementary to the mutant gene.

In yet another embodiment, any of a variety of sequencing reactions known in the art can be used to directly sequence the FCTRX gene and detect mutations by comparing the sequence of the sample FCTRX with the corresponding wild-type (control) sequence. Examples of sequencing reactions include those based on techniques developed by Maxim and Gilbert, 1977. *Proc. Natl. Acad. Sci. USA* 74: 560 or Sanger, 1977. *Proc. Natl. Acad. Sci. USA* 74: 5463. It is also contemplated that any of a variety of automated sequencing procedures can be utilized when performing the diagnostic assays (*see, e.g.,* Naeve, *et al.*, 1995. *Biotechniques* 19: 448), including sequencing by mass spectrometry (see, *e.g.*, PCT International Publication No. WO 94/16101; Cohen, *et al.*, 1996. *Adv. Chromatography* 36: 127-162; and Griffin, *et al.*, 1993. *Appl. Biochem. Biotechnol.* 38: 147-159).

Other methods for detecting mutations in the FCTRX gene include methods in which protection from cleavage agents is used to detect mismatched bases in RNA/RNA or RNA/DNA heteroduplexes. *See, e.g.,* Myers, *et al.*, 1985. *Science* 230: 1242. In general, the art technique of "mismatch cleavage" starts by providing heteroduplexes of formed by hybridizing (labeled) RNA or DNA containing the wild-type FCTRX sequence with potentially mutant RNA or DNA obtained from a tissue sample. The double-stranded duplexes are treated with an agent that cleaves single-stranded regions of the duplex such as which will exist due to basepair mismatches between the control and sample strands. For instance, RNA/DNA duplexes can be treated with RNase and DNA/DNA hybrids treated with S_1 nuclease to enzymatically digesting the mismatched regions. In other embodiments, either DNA/DNA or RNA/DNA duplexes can be treated with hydroxylamine or osmium tetroxide and with piperidine in order to digest mismatched regions. After digestion of the mismatched regions, the resulting material is then

separated by size on denaturing polyacrylamide gels to determine the site of mutation. *See, e.g.,* Cotton, *et al.,* 1988. *Proc. Natl. Acad. Sci. USA* 85: 4397; Saleeba, *et al.,* 1992. *Methods Enzymol.* 217: 286-295. In an embodiment, the control DNA or RNA can be labeled for detection.

In still another embodiment, the mismatch cleavage reaction employs one or more proteins that recognize mismatched base pairs in double-stranded DNA (so called "DNA mismatch repair" enzymes) in defined systems for detecting and mapping point mutations in FCTRX cDNAs obtained from samples of cells. For example, the mutY enzyme of *E. coli* cleaves A at G/A mismatches and the thymidine DNA glycosylase from HeLa cells cleaves T at G/T mismatches. *See, e.g.,* Hsu, *et al.*, 1994. *Carcinogenesis* 15: 1657-1662. According to an exemplary embodiment, a probe based on an FCTRX sequence, *e.g.*, a wild-type FCTRX sequence, is hybridized to a cDNA or other DNA product from a test cell(s). The duplex is treated with a DNA mismatch repair enzyme, and the cleavage products, if any, can be detected from electrophoresis protocols or the like. *See, e.g.,* U.S. Patent No. 5,459,039.

In other embodiments, alterations in electrophoretic mobility will be used to identify mutations in FCTRX genes. For example, single strand conformation polymorphism (SSCP) may be used to detect differences in electrophoretic mobility between mutant and wild type nucleic acids. *See, e.g.,* Orita, *et al.*, 1989. *Proc. Natl. Acad. Sci. USA*: 86: 2766; Cotton, 1993. *Mutat. Res.* 285: 125-144; Hayashi, 1992. *Genet. Anal. Tech. Appl.* 9: 73-79. Single-stranded DNA fragments of sample and control FCTRX nucleic acids will be denatured and allowed to renature. The secondary structure of single-stranded nucleic acids varies according to sequence, the resulting alteration in electrophoretic mobility enables the detection of even a single base change. The DNA fragments may be labeled or detected with labeled probes. The sensitivity of the assay may be enhanced by using RNA (rather than DNA), in which the secondary structure is more sensitive to a change in sequence. In one embodiment, the subject method utilizes heteroduplex analysis to separate double stranded heteroduplex molecules on the basis of changes in electrophoretic mobility. *See, e.g.,* Keen, *et al.*, 1991. *Trends Genet.* 7: 5.

In yet another embodiment, the movement of mutant or wild-type fragments in polyacrylamide gels containing a gradient of denaturant is assayed using denaturing gradient gel electrophoresis (DGGE). *See, e.g.,* Myers, *et al.,* 1985. *Nature* 313: 495. When DGGE is used as the method of analysis, DNA will be modified to insure that it does not completely denature, for example by adding a GC clamp of approximately 40 bp of high-melting GC-rich DNA by PCR. In a further embodiment, a temperature gradient is used in place of a denaturing gradient

to identify differences in the mobility of control and sample DNA. *See, e.g.,* Rosenbaum and Reissner, 1987. *Biophys. Chem.* 265: 12753.

Examples of other techniques for detecting point mutations include, but are not limited to, selective oligonucleotide hybridization, selective amplification, or selective primer extension. For example, oligonucleotide primers may be prepared in which the known mutation is placed centrally and then hybridized to target DNA under conditions that permit hybridization only if a perfect match is found. *See, e.g.,* Saiki, *et al.*, 1986. *Nature* 324: 163; Saiki, *et al.*, 1989. *Proc. Natl. Acad. Sci. USA* 86: 6230. Such allele specific oligonucleotides are hybridized to PCR amplified target DNA or a number of different mutations when the oligonucleotides are attached to the hybridizing membrane and hybridized with labeled target DNA.

Alternatively, allele specific amplification technology that depends on selective PCR amplification may be used in conjunction with the instant invention. Oligonucleotides used as primers for specific amplification may carry the mutation of interest in the center of the molecule (so that amplification depends on differential hybridization; *see, e.g.,* Gibbs, *et al.*, 1989. *Nucl. Acids Res.* 17: 2437-2448) or at the extreme 3'-terminus of one primer where, under appropriate conditions, mismatch can prevent, or reduce polymerase extension (*see, e.g.,* Prossner, 1993. *Tibtech.* 11: 238). In addition it may be desirable to introduce a novel restriction site in the region of the mutation to create cleavage-based detection. *See, e.g.,* Gasparini, *et al.,* 1992. *Mol. Cell Probes* 6: 1. It is anticipated that in certain embodiments amplification may also be performed using *Taq* ligase for amplification. *See, e.g.,* Barany, 1991. *Proc. Natl. Acad. Sci. USA* 88: 189. In such cases, ligation will occur only if there is a perfect match at the 3'-terminus of the 5' sequence, making it possible to detect the presence of a known mutation at a specific site by looking for the presence or absence of amplification.

The methods described herein may be performed, for example, by utilizing pre-packaged diagnostic kits comprising at least one probe nucleic acid or antibody reagent described herein, which may be conveniently used, *e.g.*, in clinical settings to diagnose patients exhibiting symptoms or family history of a disease or illness involving an FCTRX gene.

Furthermore, any cell type or tissue, preferably peripheral blood leukocytes, in which FCTRX is expressed may be utilized in the prognostic assays described herein. However, any biological sample containing nucleated cells may be used, including, for example, buccal mucosal cells.

Pharmacogenomics

Agents, or modulators that have a stimulatory or inhibitory effect on FCTRX activity (*e.g.*, FCTRX gene expression), as identified by a screening assay described herein can be

administered to individuals to treat (prophylactically or therapeutically) disorders (The disorders include metabolic disorders, Also within the scope of the invention is the use of a Therapeutic in the manufacture of a medicament for treating or preventing disorders or syndromes including, *e.g.*, Colorectal cancer, adenomatous polyposis coli, myelogenous leukemia, congenital ceonatal alloimmune thrombocytopenia, multiple human solid malignancies, malignant ovarian tumours particularly at the interface between epithelia and stroma, malignant brain tumors, mammary tumors, human gliomas, astrocytomas, mixed glioma/astrocytomas, renal cells carcinoma, breast adenocarcinoma, ovarian cancer, melanomas, renal cell carcinoma , clear cell and granular cell carcinomas, autocrine/paracrine stimulation of tumor cell proliferation, autocrine/paracrine stimulation of tumor cell survival and tumor cell resistance to cytotoxic therapy, paranechmal and basement membrane invasion and motility of tumor cells thereby contributing to metastasis, tumor-mediated immunosuppression of T-cell mediated immune effector cells and pathways resulting in tumor escape from immune surveilance, neurological disorders, neurodegenerative disorders, nerve trauma, familial myelodysplastic syndrome, Charcot-Marie-Tooth neuropathy, demyelinating Gardner syndrome, familial myelodysplastic syndrome; mental health conditions, immunological disorders, allergy and infection, asthma, bronchial asthma, Avellino type eosinophilia, lung diseases, reproductive disorders, male infertility, female reproductive system disorders, male and female reproductive diseases, hemangioma, deafness, glycoprotein Ia deficiency, desmoid disease, turcot syndrome, liver cirrhosis, hepatitis C, gastric disorders, pancreatic diseases like diabetes, Schistosoma mansoni infection, Spinocerebellar ataxia, Plasmodium falciparum parasitemia, Corneal dystrophy -Groenouw type I, Corneal dystrophy - lattice type I, and Reis-Bucklers corneal dystrophy) In conjunction with such treatment, the pharmacogenomics (*i.e.*, the study of the relationship between an individual's genotype and that individual's response to a foreign compound or drug) of the individual may be considered. Differences in metabolism of therapeutics can lead to severe toxicity or therapeutic failure by altering the relation between dose and blood concentration of the pharmacologically active drug. Thus, the pharmacogenomics of the individual permits the selection of effective agents (*e.g.*, drugs) for prophylactic or therapeutic treatments based on a consideration of the individual's genotype. Such pharmacogenomics can further be used to determine appropriate dosages and therapeutic regimens. Accordingly, the activity of FCTRX protein, expression of FCTRX nucleic acid, or mutation content of FCTRX genes in an individual can be determined to thereby select appropriate agent(s) for therapeutic or prophylactic treatment of the individual.

Pharmacogenomics deals with clinically significant hereditary variations in the response to drugs due to altered drug disposition and abnormal action in affected persons. See *e.g.*,

Eichelbaum, 1996. *Clin. Exp. Pharmacol. Physiol.*, 23: 983-985; Linder, 1997. *Clin. Chem.*, 43: 254-266. In general, two types of pharmacogenetic conditions can be differentiated. Genetic conditions transmitted as a single factor altering the way drugs act on the body (altered drug action) or genetic conditions transmitted as single factors altering the way the body acts on drugs (altered drug metabolism). These pharmacogenetic conditions can occur either as rare defects or as polymorphisms. For example, glucose-6-phosphate dehydrogenase (G6PD) deficiency is a common inherited enzymopathy in which the main clinical complication is hemolysis after ingestion of oxidant drugs (anti-malarials, sulfonamides, analgesics, nitrofurans) and consumption of fava beans.

As an illustrative embodiment, the activity of drug metabolizing enzymes is a major determinant of both the intensity and duration of drug action. The discovery of genetic polymorphisms of drug metabolizing enzymes (*e.g.*, N-acetyltransferase 2 (NAT 2) and cytochrome P450 enzymes CYP2D6 and CYP2C19) has provided an explanation as to why some patients do not obtain the expected drug effects or show exaggerated drug response and serious toxicity after taking the standard and safe dose of a drug. These polymorphisms are expressed in two phenotypes in the population, the extensive metabolizer (EM) and poor metabolizer (PM). The prevalence of PM is different among different populations. For example, the gene coding for CYP2D6 is highly polymorphic and several mutations have been identified in PM, which all lead to the absence of functional CYP2D6. Poor metabolizers of CYP2D6 and CYP2C19 quite frequently experience exaggerated drug response and side effects when they receive standard doses. If a metabolite is the active therapeutic moiety, PM show no therapeutic response, as demonstrated for the analgesic effect of codeine mediated by its CYP2D6-formed metabolite morphine. At the other extreme are the so called ultra-rapid metabolizers who do not respond to standard doses. Recently, the molecular basis of ultra-rapid metabolism has been identified to be due to CYP2D6 gene amplification.

Thus, the activity of FCTRX protein, expression of FCTRX nucleic acid, or mutation content of FCTRX genes in an individual can be determined to thereby select appropriate agent(s) for therapeutic or prophylactic treatment of the individual. In addition, pharmacogenetic studies can be used to apply genotyping of polymorphic alleles encoding drug-metabolizing enzymes to the identification of an individual's drug responsiveness phenotype. This knowledge, when applied to dosing or drug selection, can avoid adverse reactions or therapeutic failure and thus enhance therapeutic or prophylactic efficiency when treating a subject with an FCTRX modulator, such as a modulator identified by one of the exemplary screening assays described herein.

Monitoring of Effects During Clinical Trials

Monitoring the influence of agents (*e.g.*, drugs, compounds) on the expression or activity of FCTRX (*e.g.*, the ability to modulate aberrant cell proliferation and/or differentiation) can be applied not only in basic drug screening, but also in clinical trials. For example, the effectiveness of an agent determined by a screening assay as described herein to increase FCTRX gene expression, protein levels, or upregulate FCTRX activity, can be monitored in clinical trails of subjects exhibiting decreased FCTRX gene expression, protein levels, or downregulated FCTRX activity. Alternatively, the effectiveness of an agent determined by a screening assay to decrease FCTRX gene expression, protein levels, or downregulate FCTRX activity, can be monitored in clinical trails of subjects exhibiting increased FCTRX gene expression, protein levels, or upregulated FCTRX activity. In such clinical trials, the expression or activity of FCTRX and, preferably, other genes that have been implicated in, for example, a cellular proliferation or immune disorder can be used as a "read out" or markers of the immune responsiveness of a particular cell.

By way of example, and not of limitation, genes, including FCTRX, that are modulated in cells by treatment with an agent (*e.g.*, compound, drug or small molecule) that modulates FCTRX activity (*e.g.*, identified in a screening assay as described herein) can be identified. Thus, to study the effect of agents on cellular proliferation disorders, for example, in a clinical trial, cells can be isolated and RNA prepared and analyzed for the levels of expression of FCTRX and other genes implicated in the disorder. The levels of gene expression (*i.e.*, a gene expression pattern) can be quantified by Northern blot analysis or RT-PCR, as described herein, or alternatively by measuring the amount of protein produced, by one of the methods as described herein, or by measuring the levels of activity of FCTRX or other genes. In this manner, the gene expression pattern can serve as a marker, indicative of the physiological response of the cells to the agent. Accordingly, this response state may be determined before, and at various points during, treatment of the individual with the agent.

In one embodiment, the invention provides a method for monitoring the effectiveness of treatment of a subject with an agent (*e.g.*, an agonist, antagonist, protein, peptide, peptidomimetic, nucleic acid, small molecule, or other drug candidate identified by the screening assays described herein) comprising the steps of (*i*) obtaining a pre-administration sample from a subject prior to administration of the agent; (*ii*) detecting the level of expression of an FCTRX protein, mRNA, or genomic DNA in the preadministration sample; (*iii*) obtaining one or more post-administration samples from the subject; (*iv*) detecting the level of expression or activity of the FCTRX protein, mRNA, or genomic DNA in the post-administration samples; (*v*) comparing

the level of expression or activity of the FCTRX protein, mRNA, or genomic DNA in the pre-administration sample with the FCTRX protein, mRNA, or genomic DNA in the post administration sample or samples; and (*vi*) altering the administration of the agent to the subject accordingly. For example, increased administration of the agent may be desirable to increase the expression or activity of FCTRX to higher levels than detected, *i.e.*, to increase the effectiveness of the agent. Alternatively, decreased administration of the agent may be desirable to decrease expression or activity of FCTRX to lower levels than detected, *i.e.*, to decrease the effectiveness of the agent.

Methods of Treatment

The invention provides for both prophylactic and therapeutic methods of treating a subject at risk of (or susceptible to) a disorder or having a disorder associated with aberrant FCTRX expression or activity. The disorders include cardiomyopathy, atherosclerosis, hypertension, congenital heart defects, aortic stenosis, atrial septal defect (ASD), atrioventricular (A-V) canal defect, ductus arteriosus, pulmonary stenosis, subaortic stenosis, ventricular septal defect (VSD), valve diseases, tuberous sclerosis, scleroderma, obesity, transplantation, adrenoleukodystrophy, congenital adrenal hyperplasia, prostate cancer, neoplasm; adenocarcinoma, lymphoma, uterus cancer, fertility, hemophilia, hypercoagulation, idiopathic thrombocytopenic purpura, immunodeficiencies, graft versus host disease, AIDS, bronchial asthma, Crohn's disease; multiple sclerosis, treatment of Albright Hereditary Ostoeodystrophy, and other diseases, disorders and conditions of the like.

These methods of treatment will be discussed more fully, below.

Disease and Disorders

Diseases and disorders that are characterized by increased (relative to a subject not suffering from the disease or disorder) levels or biological activity may be treated with Therapeutics that antagonize (*i.e.*, reduce or inhibit) activity. Therapeutics that antagonize activity may be administered in a therapeutic or prophylactic manner. Therapeutics that may be utilized include, but are not limited to: (*i*) an aforementioned peptide, or analogs, derivatives, fragments or homologs thereof; (*ii*) antibodies to an aforementioned peptide; (*iii*) nucleic acids encoding an aforementioned peptide; (*iv*) administration of antisense nucleic acid and nucleic acids that are "dysfunctional" (*i.e.*, due to a heterologous insertion within the coding sequences of coding sequences to an aforementioned peptide) that are utilized to "knockout" endoggenous function of an aforementioned peptide by homologous recombination (*see*, *e.g.*, Capecchi, 1989. *Science* 244: 1288-1292); or (*v*) modulators (*i.e.*, inhibitors, agonists and antagonists, including

additional peptide mimetic of the invention or antibodies specific to a peptide of the invention) that alter the interaction between an aforementioned peptide and its binding partner.

Diseases and disorders that are characterized by decreased (relative to a subject not suffering from the disease or disorder) levels or biological activity may be treated with Therapeutics that increase (*i.e.*, are agonists to) activity. Therapeutics that upregulate activity may be administered in a therapeutic or prophylactic manner. Therapeutics that may be utilized include, but are not limited to, an aforementioned peptide, or analogs, derivatives, fragments or homologs thereof; or an agonist that increases bioavailability.

Increased or decreased levels can be readily detected by quantifying peptide and/or RNA, by obtaining a patient tissue sample (*e.g.*, from biopsy tissue) and assaying it *in vitro* for RNA or peptide levels, structure and/or activity of the expressed peptides (or mRNAs of an aforementioned peptide). Methods that are well-known within the art include, but are not limited to, immunoassays (*e.g.*, by Western blot analysis, immunoprecipitation followed by sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis, immunocytochemistry, etc.) and/or hybridization assays to detect expression of mRNAs (*e.g.*, Northern assays, dot blots, *in situ* hybridization, and the like).

Prophylactic Methods

In one aspect, the invention provides a method for preventing, in a subject, a disease or condition associated with an aberrant FCTRX expression or activity, by administering to the subject an agent that modulates FCTRX expression or at least one FCTRX activity. Subjects at risk for a disease that is caused or contributed to by aberrant FCTRX expression or activity can be identified by, for example, any or a combination of diagnostic or prognostic assays as described herein. Administration of a prophylactic agent can occur prior to the manifestation of symptoms characteristic of the FCTRX aberrancy, such that a disease or disorder is prevented or, alternatively, delayed in its progression. Depending upon the type of FCTRX aberrancy, for example, an FCTRX agonist or FCTRX antagonist agent can be used for treating the subject. The appropriate agent can be determined based on screening assays described herein. The prophylactic methods of the invention are further discussed in the following subsections.

Therapeutic Methods

Another aspect of the invention pertains to methods of modulating FCTRX expression or activity for therapeutic purposes. The modulatory method of the invention involves contacting a cell with an agent that modulates one or more of the activities of FCTRX protein activity associated with the cell. An agent that modulates FCTRX protein activity can be an agent as described herein, such as a nucleic acid or a protein, a naturally-occurring cognate ligand of an

FCTRX protein, a peptide, an FCTRX peptidomimetic, or other small molecule. In one embodiment, the agent stimulates one or more FCTRX protein activity. Examples of such stimulatory agents include active FCTRX protein and a nucleic acid molecule encoding FCTRX that has been introduced into the cell. In another embodiment, the agent inhibits one or more FCTRX protein activity. Examples of such inhibitory agents include antisense FCTRX nucleic acid molecules and anti-FCTRX antibodies. These modulatory methods can be performed *in vitro* (*e.g.*, by culturing the cell with the agent) or, alternatively, *in vivo* (*e.g.*, by administering the agent to a subject). As such, the invention provides methods of treating an individual afflicted with a disease or disorder characterized by aberrant expression or activity of an FCTRX protein or nucleic acid molecule. In one embodiment, the method involves administering an agent (*e.g.*, an agent identified by a screening assay described herein), or combination of agents that modulates (*e.g.*, up-regulates or down-regulates) FCTRX expression or activity. In another embodiment, the method involves administering an FCTRX protein or nucleic acid molecule as therapy to compensate for reduced or aberrant FCTRX expression or activity.

Stimulation of FCTRX activity is desirable in situations in which FCTRX is abnormally downregulated and/or in which increased FCTRX activity is likely to have a beneficial effect. One example of such a situation is where a subject has a disorder characterized by aberrant cell proliferation and/or differentiation (*e.g.*, cancer or immune associated disorders). Another example of such a situation is where the subject has a gestational disease (*e.g.*, preclampsia).

Determination of the Biological Effect of the Therapeutic

In various embodiments of the invention, suitable *in vitro* or *in vivo* assays are performed to determine the effect of a specific Therapeutic and whether its administration is indicated for treatment of the affected tissue.

In various specific embodiments, *in vitro* assays may be performed with representative cells of the type(s) involved in the patient's disorder, to determine if a given Therapeutic exerts the desired effect upon the cell type(s). Compounds for use in therapy may be tested in suitable animal model systems including, but not limited to rats, mice, chicken, cows, monkeys, rabbits, and the like, prior to testing in human subjects. Similarly, for *in vivo* testing, any of the animal model system known in the art may be used prior to administration to human subjects.

Prophylactic and Therapeutic Uses of the Compositions of the Invention

The FCTRX nucleic acids and proteins of the invention are useful in potential prophylactic and therapeutic applications implicated in a variety of disorders including, but not limited to: Also within the scope of the invention is the use of a Therapeutic in the manufacture

of a medicament for treating or preventing disorders or syndromes including, *e.g.*, Colorectal cancer, adenomatous polyposis coli, myelogenous leukemia, congenital ceonatal alloimmune thrombocytopenia, multiple human solid malignancies, malignant ovarian tumours particularly at the interface between epithelia and stroma, malignant brain tumors, mammary tumors, human gliomas, astrocytomas, mixed glioma/astrocytomas, renal cells carcinoma, breast adenocarcinoma, ovarian cancer, melanomas, renal cell carcinoma , clear cell and granular cell carcinomas, autocrine/paracrine stimulation of tumor cell proliferation, autocrine/paracrine stimulation of tumor cell survival and tumor cell resistance to cytotoxic therapy, paranechmal and basement membrane invasion and motility of tumor cells thereby contributing to metastasis, tumor-mediated immunosuppression of T-cell mediated immune effector cells and pathways resulting in tumor escape from immune surveilance, neurological disorders, neurodegenerative disorders, nerve trauma, familial myelodysplastic syndrome, Charcot-Marie-Tooth neuropathy, demyelinating Gardner syndrome, familial myelodysplastic syndrome; mental health conditions, immunological disorders, allergy and infection, asthma, bronchial asthma, Avellino type eosinophilia, lung diseases, reproductive disorders, male infertility, female reproductive system disorders, male and female reproductive diseases, hemangioma, deafness, glycoprotein Ia deficiency, desmoid disease, turcot syndrome, liver cirrhosis, hepatitis C, gastric disorders, pancreatic diseases like diabetes, Schistosoma mansoni infection, Spinocerebellar ataxia, Plasmodium falciparum parasitemia, Corneal dystrophy -Groenouw type I, Corneal dystrophy - lattice type I, and Reis-Bucklers corneal dystrophy.

As an example, a cDNA encoding the FCTRX protein of the invention may be useful in gene therapy, and the protein may be useful when administered to a subject in need thereof. By way of non-limiting example, the compositions of the invention will have efficacy for treatment of patients suffering from: Also within the scope of the invention is the use of a Therapeutic in the manufacture of a medicament for treating or preventing disorders or syndromes including, *e.g.*, Colorectal cancer, adenomatous polyposis coli, myelogenous leukemia, congenital ceonatal alloimmune thrombocytopenia, multiple human solid malignancies, malignant ovarian tumours particularly at the interface between epithelia and stroma, malignant brain tumors, mammary tumors, human gliomas, astrocytomas, mixed glioma/astrocytomas, renal cells carcinoma, breast adenocarcinoma, ovarian cancer, melanomas, renal cell carcinoma , clear cell and granular cell carcinomas, autocrine/paracrine stimulation of tumor cell proliferation, autocrine/paracrine stimulation of tumor cell survival and tumor cell resistance to cytotoxic therapy, paranechmal and basement membrane invasion and motility of tumor cells thereby contributing to metastasis, tumor-mediated immunosuppression of T-cell mediated immune effector cells and pathways

resulting in tumor escape from immune surveilance, neurological disorders, neurodegenerative disorders, nerve trauma, familial myelodysplastic syndrome, Charcot-Marie-Tooth neuropathy, demyelinating Gardner syndrome, familial myelodysplastic syndrome; mental health conditions, immunological disorders, allergy and infection, asthma, bronchial asthma, Avellino type eosinophilia, lung diseases, reproductive disorders, male infertility, female reproductive system disorders, male and female reproductive diseases, hemangioma, deafness, glycoprotein Ia deficiency, desmoid disease, turcot syndrome, liver cirrhosis, hepatitis C, gastric disorders, pancreatic diseases like diabetes, Schistosoma mansoni infection, Spinocerebellar ataxia, Plasmodium falciparum parasitemia, Corneal dystrophy -Groenouw type I, Corneal dystrophy - lattice type I, and Reis-Bucklers corneal dystrophy.

Both the novel nucleic acid encoding the FCTRX protein, and the FCTRX protein of the invention, or fragments thereof, may also be useful in diagnostic applications, wherein the presence or amount of the nucleic acid or the protein are to be assessed. A further use could be as an anti-bacterial molecule (*i.e.*, some peptides have been found to possess anti-bacterial properties). These materials are further useful in the generation of antibodies which immunospecifically-bind to the novel substances of the invention for use in therapeutic or diagnostic methods.

EXAMPLES

The following examples illustrate by way of non-limiting example various aspects of the invention.

The following examples illustrate by way of non-limiting example various aspects of the invention.

Example 1: Method of Identifying the Nucleic Acids

The novel nucleic acids of the invention were identified by TblastN using a proprietary sequence file, run against the Genomic Daily Files made available by GenBank. The nucleic acids were further predicted by the proprietary software program GenScan™, including selection of exons. These were further modified by means of similarities using BLAST searches. The sequences were then manually corrected for apparent inconsistencies, thereby obtaining the sequences encoding the full-length proteins.

Example 2. Quantitative expression analysis of FCTR2 in various cells and tissues

The quantitative expression of various clones was assessed using microtiter plates containing RNA samples from a variety of normal and pathology-derived cells, cell lines and tissues using real time quantitative PCR (RTQ PCR; TAQMAN®). RTQ PCR was performed on a Perkin-Elmer Biosystems ABI PRISM® 7700 Sequence Detection System. Various collections of samples are assembled on the plates, and referred to as Panel 1 (containing cells and cell lines from normal and cancer sources), Panel 2 (containing samples derived from tissues, in particular from surgical samples, from normal and cancer sources), Panel 3 (containing samples derived from a wide variety of cancer sources) and Panel 4 (containing cells and cell lines from normal cells and cells related to inflammatory conditions).

First, the RNA samples were normalized to constitutively expressed genes such as β-actin and GAPDH. RNA (~50 ng total or ~1 ng polyA+) was converted to cDNA using the TAQMAN® Reverse Transcription Reagents Kit (PE Biosystems, Foster City, CA; Catalog No. N808-0234) and random hexamers according to the manufacturer's protocol. Reactions were performed in 20 ul and incubated for 30 min. at 48^{0}C. cDNA (5 ul) was then transferred to a separate plate for the TAQMAN® reaction using β-actin and GAPDH TAQMAN® Assay Reagents (PE Biosystems; Catalog Nos. 4310881E and 4310884E, respectively) and TAQMAN® universal PCR Master Mix (PE Biosystems; Catalog No. 4304447) according to the manufacturer's protocol. Reactions were performed in 25 ul using the following parameters: 2 min. at 50^{0}C; 10 min. at 95^{0}C; 15 sec. at 95^{0}C/1 min. at 60^{0}C (40 cycles). Results were recorded as CT values (cycle at which a given sample crosses a threshold level of fluorescence) using a log scale, with the difference in RNA concentration between a given sample and the sample with the lowest CT value being represented as 2 to the power of delta CT. The percent relative expression is then obtained by taking the reciprocal of this RNA difference and multiplying by 100. The average CT values obtained for ß-actin and GAPDH were used to normalize RNA samples. The RNA sample generating the highest CT value required no further diluting, while all other samples were diluted relative to this sample according to their β-actin /GAPDH average CT values.

Normalized RNA (5 ul) was converted to cDNA and analyzed via TAQMAN® using One Step RT-PCR Master Mix Reagents (PE Biosystems; Catalog No. 4309169) and gene-specific primers according to the manufacturer's instructions. Probes and primers were designed for each assay according to Perkin Elmer Biosystem's *Primer Express* Software package (version I for Apple Computer's Macintosh Power PC) or a similar algorithm using the target sequence as input. Default settings were used for reaction conditions and the following

parameters were set before selecting primers: primer concentration = 250 nM, primer melting temperature (T_m) range = 58°-60° C, primer optimal Tm = 59° C, maximum primer difference = 2° C, probe does not have 5' G, probe T_m must be 10° C greater than primer T_m, amplicon size 75 bp to 100 bp. The probes and primers selected (see below) were synthesized by Synthegen (Houston, TX, USA). Probes were double purified by HPLC to remove uncoupled dye and evaluated by mass spectroscopy to verify coupling of reporter and quencher dyes to the 5' and 3' ends of the probe, respectively. Their final concentrations were: forward and reverse primers, 900 nM each, and probe, 200nM.

PCR conditions: Normalized RNA from each tissue and each cell line was spotted in each well of a 96 well PCR plate (Perkin Elmer Biosystems). PCR cocktails including two probes (a probe specific for the target clone and another gene-specific probe multiplexed with the target probe) were set up using 1X TaqMan™ PCR Master Mix for the PE Biosystems 7700, with 5 mM MgCl2, dNTPs (dA, G, C, U at 1:1:1:2 ratios), 0.25 U/ml AmpliTaq Gold™ (PE Biosystems), and 0.4 U/μl RNase inhibitor, and 0.25 U/μl reverse transcriptase. Reverse transcription was performed at 48° C for 30 minutes followed by amplification/PCR cycles as follows: 95° C 10 min, then 40 cycles of 95° C for 15 seconds, 60° C for 1 minute.

In the results for Panel 1, the following abbreviations are used:

ca. = carcinoma,

* = established from metastasis,

met = metastasis,

s cell var= small cell variant,

non-s = non-sm =non-small,

squam = squamous,

pl. eff = pl effusion = pleural effusion,

glio = glioma,

astro = astrocytoma, and

neuro = neuroblastoma.

Panel 2

The plates for Panel 2 generally include 2 control wells and 94 test samples composed of RNA or cDNA isolated from human tissue procured by surgeons working in close cooperation

with the National Cancer Institute's Cooperative Human Tissue Network (CHTN) or the National Disease Research Initiative (NDRI). The tissues are derived from human malignancies and in cases where indicated many malignant tissues have "matched margins" obtained from noncancerous tissue just adjacent to the tumor. These are termed normal adjacent tissues and are denoted "NAT" in the results below. The tumor tissue and the "matched margins" are evaluated by two independent pathologists (the surgical pathologists and again by a pathologists at NDRI or CHTN). This analysis provides a gross histopathological assessment of tumor differentiation grade. Moreover, most samples include the original surgical pathology report that provides information regarding the clinical stage of the patient. These matched margins are taken from the tissue surrounding (i.e. immediately proximal) to the zone of surgery (designated "NAT", for normal adjacent tissue, in Table RR). In addition, RNA and cDNA samples were obtained from various human tissues derived from autopsies performed on elderly people or sudden death victims (accidents, etc.). These tissue were ascertained to be free of disease and were purchased from various commercial sources such as Clontech (Palo Alto, CA), Research Genetics, and Invitrogen.

RNA integrity from all samples is controlled for quality by visual assessment of agarose gel electropherograms using 28S and 18S ribosomal RNA staining intensity ratio as a guide (2:1 to 2.5:1 28s:18s) and the absence of low molecular weight RNAs that would be indicative of degradation products. Samples are controlled against genomic DNA contamination by RTQ PCR reactions run in the absence of reverse transcriptase using probe and primer sets designed to amplify across the span of a single exon.

Panel 4

Panel 4 includes samples on a 96 well plate (2 control wells, 94 test samples) composed of RNA (Panel 4r) or cDNA (Panel 4d) isolated from various human cell lines or tissues related to inflammatory conditions. Total RNA from control normal tissues such as colon and lung (Stratagene ,La Jolla, CA) and thymus and kidney (Clontech) were employed. Total RNA from liver tissue from cirrhosis patients and kidney from lupus patients was obtained from BioChain (Biochain Institute, Inc., Hayward, CA). Intestinal tissue for RNA preparation from patients diagnosed as having Crohn's disease and ulcerative colitis was obtained from the National Disease Research Interchange (NDRI) (Philadelphia, PA).

Astrocytes, lung fibroblasts, dermal fibroblasts, coronary artery smooth muscle cells, small airway epithelium, bronchial epithelium, microvascular dermal endothelial cells, microvascular lung endothelial cells, human pulmonary aortic endothelial cells, human umbilical vein endothelial cells were all purchased from Clonetics (Walkersville, MD) and grown in the media supplied for these cell types by Clonetics. These primary cell types were activated with various cytokines or combinations of cytokines for 6 and/or 12-14 hours, as indicated. The following cytokines were used; IL-1 beta at approximately 1-5 ng/ml, TNF alpha at approximately 5-10 ng/ml, IFN gamma at approximately 20-50 ng/ml, IL-4 at approximately 5-10 ng/ml, IL-9 at approximately 5-10 ng/ml, IL-13 at approximately 5-10 ng/ml. Endothelial cells were sometimes starved for various times by culture in the basal media from Clonetics with 0.1% serum.

Mononuclear cells were prepared from blood of employees at CuraGen Corporation, using Ficoll. LAK cells were prepared from these cells by culture in DMEM 5% FCS (Hyclone), 100 μM non essential amino acids (Gibco/Life Technologies, Rockville, MD), 1 mM sodium pyruvate (Gibco), mercaptoethanol 5.5 x 10^{-5} M (Gibco), and 10 mM Hepes (Gibco) and Interleukin 2 for 4-6 days. Cells were then either activated with 10-20 ng/ml PMA and 1-2 μg/ml ionomycin, IL-12 at 5-10 ng/ml, IFN gamma at 20-50 ng/ml and IL-18 at 5-10 ng/ml for 6 hours. In some cases, mononuclear cells were cultured for 4-5 days in DMEM 5% FCS (Hyclone), 100 μM non essential amino acids (Gibco), 1 mM sodium pyruvate (Gibco), mercaptoethanol 5.5 x 10^{-5} M (Gibco), and 10 mM Hepes (Gibco) with PHA (phytohemagglutinin) or PWM (pokeweed mitogen) at approximately 5 μg/ml. Samples were taken at 24, 48 and 72 hours for RNA preparation. MLR (mixed lymphocyte reaction) samples were obtained by taking blood from two donors, isolating the mononuclear cells using Ficoll and mixing the isolated mononuclear cells 1:1 at a final concentration of approximately $2x10^6$ cells/ml in DMEM 5% FCS (Hyclone), 100 μM non essential amino acids (Gibco), 1 mM sodium pyruvate (Gibco), mercaptoethanol (5.5 x 10^{-5} M) (Gibco), and 10 mM Hepes (Gibco). The MLR was cultured and samples taken at various time points ranging from 1- 7 days for RNA preparation.

Monocytes were isolated from mononuclear cells using CD14 Miltenyi Beads, **+ve** VS selection columns and a Vario Magnet according to the manufacturer's instructions. Monocytes were differentiated into dendritic cells by culture in DMEM 5% fetal calf serum (FCS) (Hyclone, Logan, UT), 100 μM non essential amino acids (Gibco), 1 mM sodium pyruvate

(Gibco), mercaptoethanol 5.5 x 10^{-5} M (Gibco), and 10 mM Hepes (Gibco), 50 ng/ml GMCSF and 5 ng/ml IL-4 for 5-7 days. Macrophages were prepared by culture of monocytes for 5-7 days in DMEM 5% FCS (Hyclone), 100 μM non essential amino acids (Gibco), 1 mM sodium pyruvate (Gibco), mercaptoethanol 5.5 x 10^{-5} M (Gibco), 10 mM Hepes (Gibco) and 10% AB Human Serum or MCSF at approximately 50 ng/ml. Monocytes, macrophages and dendritic cells were stimulated for 6 and 12-14 hours with lipopolysaccharide (LPS) at 100 ng/ml. Dendritic cells were also stimulated with anti-CD40 monoclonal antibody (Pharmingen) at 10 μg/ml for 6 and 12-14 hours.

CD4 lymphocytes, CD8 lymphocytes and NK cells were also isolated from mononuclear cells using CD4, CD8 and CD56 Miltenyi beads, positive VS selection columns and a Vario Magnet according to the manufacturer's instructions. CD45RA and CD45RO CD4 lymphocytes were isolated by depleting mononuclear cells of CD8, CD56, CD14 and CD19 cells using CD8, CD56, CD14 and CD19 Miltenyi beads and +ve selection. Then CD45RO beads were used to isolate the CD45RO CD4 lymphocytes with the remaining cells being CD45RA CD4 lymphocytes. CD45RA CD4, CD45RO CD4 and CD8 lymphocytes were placed in DMEM 5% FCS (Hyclone), 100 μM non essential amino acids (Gibco), 1 mM sodium pyruvate (Gibco), mercaptoethanol 5.5 x 10^{-5} M (Gibco), and 10 mM Hepes (Gibco) and plated at 10^6 cells/ml onto Falcon 6 well tissue culture plates that had been coated overnight with 0.5 μg/ml anti-CD28 (Pharmingen) and 3 ug/ml anti-CD3 (OKT3, ATCC) in PBS. After 6 and 24 hours, the cells were harvested for RNA preparation. To prepare chronically activated CD8 lymphocytes, we activated the isolated CD8 lymphocytes for 4 days on anti-CD28 and anti-CD3 coated plates and then harvested the cells and expanded them in DMEM 5% FCS (Hyclone), 100 μM non essential amino acids (Gibco), 1 mM sodium pyruvate (Gibco), mercaptoethanol 5.5 x 10^{-5} M (Gibco), and 10 mM Hepes (Gibco) and IL-2. The expanded CD8 cells were then activated again with plate bound anti-CD3 and anti-CD28 for 4 days and expanded as before. RNA was isolated 6 and 24 hours after the second activation and after 4 days of the second expansion culture. The isolated NK cells were cultured in DMEM 5% FCS (Hyclone), 100 μM non essential amino acids (Gibco), 1 mM sodium pyruvate (Gibco), mercaptoethanol 5.5 x 10^{-5} M (Gibco), and 10 mM Hepes (Gibco) and IL-2 for 4-6 days before RNA was prepared.

To obtain B cells, tonsils were procured from NDRI. The tonsil was cut up with sterile dissecting scissors and then passed through a sieve. Tonsil cells were then spun down and resupended at 10^6 cells/ml in DMEM 5% FCS (Hyclone), 100 μM non essential amino acids

(Gibco), 1 mM sodium pyruvate (Gibco), mercaptoethanol 5.5 x 10^{-5} M (Gibco), and 10 mM Hepes (Gibco). To activate the cells, we used PWM at 5 μg/ml or anti-CD40 (Pharmingen) at approximately 10 μg/ml and IL-4 at 5-10 ng/ml. Cells were harvested for RNA preparation at 24,48 and 72 hours.

To prepare the primary and secondary Th1/Th2 and Tr1 cells, six-well Falcon plates were coated overnight with 10 μg/ml anti-CD28 (Pharmingen) and 2 μg/ml OKT3 (ATCC), and then washed twice with PBS. Umbilical cord blood CD4 lymphocytes (Poietic Systems, German Town, MD) were cultured at 10^{5}-10^{6} cells/ml in DMEM 5% FCS (Hyclone), 100 μM non essential amino acids (Gibco), 1 mM sodium pyruvate (Gibco), mercaptoethanol 5.5 x 10^{-5} M (Gibco), 10 mM Hepes (Gibco) and IL-2 (4 ng/ml). IL-12 (5 ng/ml) and anti-IL4 (1 μg/ml) were used to direct to Th1, while IL-4 (5 ng/ml) and anti-IFN gamma (1 μg/ml) were used to direct to Th2 and IL-10 at 5 ng/ml was used to direct to Tr1. After 4-5 days, the activated Th1, Th2 and Tr1 lymphocytes were washed once in DMEM and expanded for 4-7 days in DMEM 5% FCS (Hyclone), 100 μM non essential amino acids (Gibco), 1 mM sodium pyruvate (Gibco), mercaptoethanol 5.5 x 10^{-5} M (Gibco), 10 mM Hepes (Gibco) and IL-2 (1 ng/ml). Following this, the activated Th1, Th2 and Tr1 lymphocytes were re-stimulated for 5 days with anti-CD28/OKT3 and cytokines as described above, but with the addition of anti-CD95L (1 μg/ml) to prevent apoptosis. After 4-5 days, the Th1, Th2 and Tr1 lymphocytes were washed and then expanded again with IL-2 for 4-7 days. Activated Th1 and Th2 lymphocytes were maintained in this way for a maximum of three cycles. RNA was prepared from primary and secondary Th1, Th2 and Tr1 after 6 and 24 hours following the second and third activations with plate bound anti-CD3 and anti-CD28 mAbs and 4 days into the second and third expansion cultures in Interleukin 2.

The following leukocyte cells lines were obtained from the ATCC: Ramos, EOL-1, KU-812. EOL cells were further differentiated by culture in 0.1 mM dbcAMP at 5 x10^{5} cells/ml for 8 days, changing the media every 3 days and adjusting the cell concentration to 5 x10^{5} cells/ml. For the culture of these cells, we used DMEM or RPMI (as recommended by the ATCC), with the addition of 5% FCS (Hyclone), 100 μM non essential amino acids (Gibco), 1 mM sodium pyruvate (Gibco), mercaptoethanol 5.5 x 10^{-5} M (Gibco), 10 mM Hepes (Gibco). RNA was either prepared from resting cells or cells activated with PMA at 10 ng/ml and ionomycin at 1 μg/ml for 6 and 14 hours. Keratinocyte line CCD106 and an airway epithelial tumor line NCI-

H292 were also obtained from the ATCC. Both were cultured in DMEM 5% FCS (Hyclone), 100 µM non essential amino acids (Gibco), 1 mM sodium pyruvate (Gibco), mercaptoethanol 5.5 x 10^{-5} M (Gibco), and 10 mM Hepes (Gibco). CCD1106 cells were activated for 6 and 14 hours with approximately 5 ng/ml TNF alpha and 1 ng/ml IL-1 beta, while NCI-H292 cells were activated for 6 and 14 hours with the following cytokines: 5 ng/ml IL-4, 5 ng/ml IL-9, 5 ng/ml IL-13 and 25 ng/ml IFN gamma.

For these cell lines and blood cells, RNA was prepared by lysing approximately 10^7 cells/ml using Trizol (Gibco BRL). Briefly, 1/10 volume of bromochloropropane (Molecular Research Corporation) was added to the RNA sample, vortexed and after 10 minutes at room temperature, the tubes were spun at 14,000 rpm in a Sorvall SS34 rotor. The aqueous phase was removed and placed in a 15 ml Falcon Tube. An equal volume of isopropanol was added and left at –20 degrees C overnight. The precipitated RNA was spun down at 9,000 rpm for 15 min in a Sorvall SS34 rotor and washed in 70% ethanol. The pellet was redissolved in 300 µl of RNAse-free water and 35 µl buffer (Promega) 5 µl DTT, 7 µl RNAsin and 8 µl DNAse were added. The tube was incubated at 37 degrees C for 30 minutes to remove contaminating genomic DNA, extracted once with phenol chloroform and re-precipitated with 1/10 volume of 3 M sodium acetate and 2 volumes of 100% ethanol. The RNA was spun down and placed in RNAse free water. RNA was stored at –80 degrees C.

The above detailed procedures were carried out to obtain the taqman profiles of the clones in question.

Given below are the Primers and the Taqman results for the following clones:

58092213.0.36 – Probe Name: Ag809 (Table 9 and Table 10)

29692275.0.1 – Probe Name: Ag2773 (Table 11 and Table 12)

32125243.0.21 – Probe Name: Ag427 (Table 13 and Table 14)

27455183.0.19 – Probe Name: Ag1541 (Table 15 and Table 16, 17, 18)

Table 8: Primer Design for Probe Ag809 (FCTR1)

Primer	Sequences	TM	Length	Start Po	SEQID NO
Forward	5'-ATGTGATCTTTGGCTGTGAAGT-3'	58.7	22	337	24
Probe	FAM-5'-CTACCCCATGGCCTCCATCGAGT-3'-TAMRA	69.4	23	365	25
Reverse		59.9	19	393	26

	5'-GGATGTCCAAGCCATCCTT-3'				

TABLE 9: TAQMAN RESULTS FOR FCTR1

Tissue_Name	Panel 1	Tissue_Name	Panel 2D	Tissue_Name	Panel 4D
Liver adenocarcinoma	79.6	Normal Colon GENPAK 061003	6.8	93768_Secondary Th1_anti-CD28/anti-CD3	2.0
Heart (fetal)	43.8	83219 CC Well to Mod Diff (ODO3866)	6.1	93769_Secondary Th2_anti-CD28/anti-CD3	1.5
Pancreas	2.1	83220 CC NAT (ODO3866)	2.5	93770_Secondary Tr1_anti-CD28/anti-CD3	2.5
Pancreatic ca. CAPAN 2	4.7	83221 CC Gr.2 rectosigmoid (ODO3868)	0.9	93573_Secondary Th1_resting day 4-6 in IL-2	1.0
Adrenal gland	2.3	83222 CC NAT (ODO3868)	1.2	93572_Secondary Th2_resting day 4-6 in IL-2	3.0
Thyroid	6.5	83235 CC Mod Diff (ODO3920)	3.8	93571_Secondary Tr1_resting day 4-6 in IL-2	1.7
Salivary gland	12.3	83236 CC NAT (ODO3920)	1.3	93568_primary Th1_anti-CD28/anti-CD3	0.4
Pituitary gland	8.7	83237 CC Gr.2 ascend colon (ODO3921)	6.9	93569_primary Th2_anti-CD28/anti-CD3	1.5
Brain (fetal)	0.0	83238 CC NAT (ODO3921)	4.0	93570_primary Tr1_anti-CD28/anti-CD3	2.0
Brain (whole)	3.0	83241 CC from Partial Hepatectomy (ODO4309)	1.2	93565_primary Th1_resting dy 4-6 in IL-2	5.4
Brain (amygdala)	2.4	83242 Liver NAT (ODO4309)	0.6	93566_primary Th2_resting dy 4-6 in IL-2	3.1
Brain (cerebellum)	0.0	87472 Colon mets to lung (OD04451-01)	4.4	93567_primary Tr1_resting dy 4-6 in IL-2	0.0
Brain (hippocampus)	13.0	87473 Lung NAT (OD04451-02)	1.2	93351_CD45RA CD4 lymphocyte_anti-CD28/anti-CD3	11.2
Brain (thalamus)	3.0	Normal Prostate Clontech A+ 6546-1	10.2	93352_CD45RO CD4 lymphocyte_anti-CD28/anti-CD3	1.2
Cerebral Cortex	2.3	84140 Prostate Cancer (OD04410)	41.8	93251_CD8 Lymphocytes_anti-CD28/anti-CD3	0.9
Spinal cord	2.6	84141 Prostate NAT (OD04410)	25.7	93353_chronic CD8 Lymphocytes 2ry_resting dy 4-6 in IL-2	0.0
CNS ca. (glio/astro) U87-MG	12.1	87073 Prostate Cancer (OD04720-01)	11.0	93574_chronic CD8 Lymphocytes 2ry_activated CD3/CD28	0.6
CNS ca. (glio/astro) U-118-MG	100.0	87074 Prostate NAT (OD04720-02)	10.0	93354_CD4_none	1.1
CNS ca. (astro) SW1783	6.5	Normal Lung GENPAK 061010	7.9	93252_Secondary Th1/Th2/Tr1_anti-CD95 CH11	0.0
CNS ca.* (neuro; met) SK-N-AS	52.1	83239 Lung Met to Muscle	6.5	93103_LAK cells_resting	0.5

		(ODO4286)			
CNS ca. (astro) SF-539	12.6	83240 Muscle NAT (ODO4286)	2.6	93788_LAK cells_IL-2	0.0
CNS ca. (astro) SNB-75	11.9	84136 Lung Malignant Cancer (OD03126)	14.8	93787_LAK cells_IL-2+IL-12	0.7
CNS ca. (glio)SNB-19	0.0	84137 Lung NAT (OD03126)	3.2	93789_LAK cells_IL-2+IFN gamma	1.1
CNS ca. (glio)U251	0.9	84871 Lung Cancer (OD04404)	2.1	93790_LAK cells_IL-2+ IL-18	0.3
CNS ca. (glio) SF-295	12.6	84872 Lung NAT (OD04404)	1.9	93104_LAK cells_PMA/ionomycin and IL-18	0.0
Heart	13.9	84875 Lung Cancer (OD04565)	0.3	93578_NK Cells IL-2_resting	1.3
Skeletal muscle	3.2	85950 Lung Cancer (OD04237-01)	1.3	93109_Mixed Lymphocyte Reaction_Two Way MLR	0.5
Bone marrow	3.6	85970 Lung NAT (OD04237-02)	2.6	93110_Mixed Lymphocyte Reaction_Two Way MLR	0.5
Thymus	4.2	83255 Ocular Mel Met to Liver (ODO4310)	0.1	93111_Mixed Lymphocyte Reaction_Two Way MLR	2.7
Spleen	61.6	83256 Liver NAT (ODO4310)	0.6	93112_Mononuclear Cells (PBMCs)_resting	0.0
Lymph node	3.3	84139 Melanoma Mets to Lung (OD04321)	2.5	93113_Mononuclear Cells (PBMCs)_PWM	1.3
Colorectal	11.9	84138 Lung NAT (OD04321)	2.6	93114_Mononuclear Cells (PBMCs)_PHA-L	1.0
Stomach	28.3	Normal Kidney GENPAK 061008	5.6	93249_Ramos (B cell)_none	1.2
Small intestine	4.5	83786 Kidney Ca, Nuclear grade 2 (OD04338)	0.6	93250_Ramos (B cell)_ionomycin	2.3
Colon ca. SW480	46.7	83787 Kidney NAT (OD04338)	3.7	93349_B lymphocytes_PWM	4.3
Colon ca.* (SW480 met)SW620	19.0	83788 Kidney Ca Nuclear grade 1/2 (OD04339)	0.8	93350_B lymphoytes_CD40L and IL-4	1.4
Colon ca. HT29	5.3	83789 Kidney NAT (OD04339)	3.1	92665_EOL-1 (Eosinophil)_dbcAMP differentiated	7.2
Colon ca. HCT-116	5.0	83790 Kidney Ca, Clear cell type (OD04340)	1.5	93248_EOL-1 (Eosinophil)_dbcAMP/PMAionomycin	3.0
Colon ca. CaCo-2	49.3	83791 Kidney NAT (OD04340)	5.1	93356_Dendritic Cells_none	1.5
83219 CC Well to Mod Diff (ODO3866)	3.0	83792 Kidney Ca, Nuclear grade 3 (OD04348)	14.5	93355_Dendritic Cells_LPS 100 ng/ml	0.7
Colon ca. HCC-2998	27.7	83793 Kidney NAT (OD04348)	2.5	93775_Dendritic Cells_anti-CD40	0.5
Gastric ca.* (liver met) NCI-N87	10.5	87474 Kidney Cancer	1.7	93774_Monocytes_resting	0.5

		(OD04622-01)			
Bladder	3.7	87475 Kidney NAT (OD04622-03)	2.0	93776_Monocytes_LPS 50 ng/ml	0.0
Trachea	23.5	85973 Kidney Cancer (OD04450-01)	0.3	93581_Macrophages_resting	1.3
Kidney	1.8	85974 Kidney NAT (OD04450-03)	2.0	93582_Macrophages_LPS 100 ng/ml	1.8
Kidney (fetal)	1.9	Kidney Cancer Clontech 8120607	7.0	93098_HUVEC (Endothelial)_none	2.3
Renal ca. 786-0	7.0	Kidney NAT Clontech 8120608	1.5	93099_HUVEC (Endothelial)_starved	9.0
Renal ca. A498	6.8	Kidney Cancer Clontech 8120613	2.0	93100_HUVEC (Endothelial)_IL-1b	1.2
Renal ca.RXF 393	4.7	Kidney NAT Clontech 8120614	4.1	93779_HUVEC (Endothelial)_IFN gamma	1.4
Renal ca.ACHN	9.8	Kidney Cancer Clontech 9010320	2.2	93102_HUVEC (Endothelial)_TNF alpha + IFN gamma	0.8
Renal ca.UO-31	1.3	Kidney NAT Clontech 9010321	3.5	93101_HUVEC (Endothelial)_TNF alpha + IL4	1.1
Renal ca.TK-10	0.6	Normal Uterus GENPAK 061018	3.1	93781_HUVEC (Endothelial)_IL-11	3.0
Liver	0.8	Uterus Cancer GENPAK 064011	17.6	93583_Lung Microvascular Endothelial Cells_none	0.8
Liver (fetal)	1.1	Normal Thyroid Clontech A+ 6570-1	3.7	93584_Lung Microvascular Endothelial Cells_TNFa (4 ng/ml) and IL1b (1 ng/ml)	0.5
Liver ca. (hepatoblast) HepG2	54.0	Thyroid Cancer GENPAK 064010	1.2	92662_Microvascular Dermal endothelium_none	1.1
Lung	3.9	Thyroid Cancer INVITROGEN A302152	0.6	92663_Microsvasular Dermal endothelium_TNFa (4 ng/ml) and IL1b (1 ng/ml)	1.0
Lung (fetal)	9.0	Thyroid NAT INVITROGEN A302153	2.6	93773_Bronchial epithelium_TNFa (4 ng/ml) and IL1b (1 ng/ml) **	0.0
Lung ca. (small cell) LX-1	34.4	Normal Breast GENPAK 061019	3.4	93347_Small Airway Epithelium_none	0.4
Lung ca. (small cell) NCI-H69	3.0	84877 Breast Cancer (OD04566)	0.9	93348_Small Airway Epithelium_TNFa (4 ng/ml) and IL1b (1 ng/ml)	0.5
Lung ca. (s.cell var.) SHP-77	13.0	85975 Breast Cancer (OD04590-01)	67.8	92668_Coronery Artery SMC_resting	5.8
Lung ca. (large cell)NCI-H460	6.8	85976 Breast Cancer Mets (OD04590-03)	51.1	92669_Coronery Artery SMC_TNFa (4 ng/ml) and IL1b (1 ng/ml)	2.3
Lung ca. (non-sm. cell) A549	3.4	87070 Breast Cancer Metastasis	12.7	93107_astrocytes_resting	2.7

		(OD04655-05)			
Lung ca. (non-s.cell) NCI-H23	34.4	GENPAK Breast Cancer 064006	8.9	93108_astrocytes_TNFa (4 ng/ml) and IL1b (1 ng/ml)	0.0
Lung ca (non-s.cell) HOP-62	10.5	Breast Cancer Clontech 9100266	6.2	92666_KU-812 (Basophil)_resting	6.8
Lung ca. (non-s.cl) NCI-H522	47.6	Breast NAT Clontech 9100265	3.3	92667_KU-812 (Basophil)_PMA/ionoycin	8.4
Lung ca. (squam.) SW 900	4.7	Breast Cancer INVITROGEN A209073	3.4	93579_CCD1106 (Keratinocytes)_none	1.6
Lung ca. (squam.) NCI-H596	0.7	Breast NAT INVITROGEN A2090734	8.7	93580_CCD1106 (Keratinocytes)_TNFa and IFNg **	1.4
Mammary gland	9.9	Normal Liver GENPAK 061009	1.1	93791_Liver Cirrhosis	4.2
Breast ca.* (pl. effusion) MCF-7	5.6	Liver Cancer GENPAK 064003	0.6	93792_Lupus Kidney	1.9
Breast ca.* (pl.ef) MDA-MB-231	21.3	Liver Cancer Research Genetics RNA 1025	0.6	93577_NCI-H292	39.5
Breast ca.* (pl. effusion) T47D	66.0	Liver Cancer Research Genetics RNA 1026	1.4	93358_NCI-H292_IL-4	39.0
Breast ca. BT-549	7.6	Paired Liver Cancer Tissue Research Genetics RNA 6004-T	1.3	93360_NCI-H292_IL-9	65.5
Breast ca.MDA-N	18.7	Paired Liver Tissue Research Genetics RNA 6004-N	1.3	93359_NCI-H292_IL-13	37.1
Ovary	12.1	Paired Liver Cancer Tissue Research Genetics RNA 6005-T	1.1	93357_NCI-H292_IFN gamma	31.9
Ovarian ca.OVCAR-3	3.5	Paired Liver Tissue Research Genetics RNA 6005-N	0.3	93777_HPAEC_-	0.5
Ovarian ca.OVCAR-4	4.0	Normal Bladder GENPAK 061001	5.9	93778_HPAEC_IL-1 beta/TNA alpha	1.2
Ovarian ca. OVCAR-5	9.1	Bladder Cancer Research Genetics RNA 1023	1.7	93254_Normal Human Lung Fibroblast_none	42.3
Ovarian ca. OVCAR-8	12.7	Bladder Cancer INVITROGEN A302173	1.9	93253_Normal Human Lung Fibroblast_TNFa (4 ng/ml) and IL-1b (1 ng/ml)	17.8
Ovarian ca.IGROV-1	9.8	87071 Bladder Cancer (OD04718-01)	2.0	93257_Normal Human Lung Fibroblast_IL-4	100.0
Ovarian ca.*	0.4	87072 Bladder	3.3	93256_Normal Human Lung	72.7

(ascites) SK-OV-3		Normal Adjacent (OD04718-03)		Fibroblast_IL-9	
Uterus	6.9	Normal Ovary Res. Gen.	2.2	93255_Normal Human Lung Fibroblast_IL-13	60.7
Plancenta	4.6	Ovarian Cancer GENPAK 064008	29.1	93258_Normal Human Lung Fibroblast_IFN gamma	81.8
Prostate	15.7	87492 Ovary Cancer (OD04768-07)	100.0	93106_Dermal Fibroblasts CCD1070_resting	76.8
Prostate ca.* (bone met)PC-3	35.9	87493 Ovary NAT (OD04768-08)	2.2	93361_Dermal Fibroblasts CCD1070_TNF alpha 4 ng/ml	30.2
Testis	14.6	Normal Stomach GENPAK 061017	13.1	93105_Dermal Fibroblasts CCD1070_IL-1 beta 1 ng/ml	38.2
Melanoma Hs688(A).T	13.5	NAT Stomach Clontech 9060359	8.8	93772_dermal fibroblast_IFN gamma	34.2
Melanoma* (met) Hs688(B).T	71.2	Gastric Cancer Clontech 9060395	2.5	93771_dermal fibroblast_IL-4	80.7
Melanoma UACC-62	1.7	NAT Stomach Clontech 9060394	9.7	93259_IBD Colitis 1**	0.0
Melanoma M14	9.5	Gastric Cancer Clontech 9060397	15.9	93260_IBD Colitis 2	0.3
Melanoma LOX IMVI	2.4	NAT Stomach Clontech 9060396	12.9	93261_IBD Crohns	1.4
Melanoma* (met)SK-MEL-5	3.4	Gastric Cancer GENPAK 064005	12.1	735010_Colon_normal	35.6
Adipose	5.9			735019_Lung_none	11.0
				64028-1_Thymus_none	5.8
				64030-1_Kidney_none	9.7

Taqman results shown in Table 9 demonstrates that cFCTR1 is highly expressed by tumor cell lines and also overexpressed in tumor tissues, specifically breast and ovarian tumor compared to Normal Adjacent Tissues (NAT). There are reports that follistatin can act as a modulator of tumor growth and its expression also correlate with polycystic ovary syndrome, a benign form of ovarian tumor.

Table 10: Primer Design for Probe Ag2773 (FCTR4)

Primer	Sequences	TM	Length	Start Po	SEQ ID NO
Forward	5'-CCTTGCTTTGTCATATGCTGTT-3'	59.3	22	243	29
Probe	FAM-5'-CCCTTTGCCTGGAATATAAACTCTCA-3'-TAMRA	64.6	26	265	30
Reverse	5'-AGAGGAAGCTTTCTGGAGAAGA-3'	58.9	22	313	31

TABLE 11: TAQMAN RESULTS FOR CLONE FCTR4

Tissue_Name	Panel 1D	Tissue_Name	Panel 2D	Tissue_Name	Panel 4D
Liver adenocarcinoma	18.3	Normal Colon GENPAK 061003	41.2	93768_Secondary Th1_anti-CD28/anti-CD3	12.7
Heart (fetal)	4.3	83219 CC Well to Mod Diff (ODO3866)	5.2	93769_Secondary Th2_anti-CD28/anti-CD3	14.2
Pancreas	3.1	83220 CC NAT (ODO3866)	2.5	93770_Secondary Tr1_anti-CD28/anti-CD3	14.7
Pancreatic ca.CAPAN 2	20.0	83221 CC Gr.2 rectosigmoid (ODO3868)	0.7	93573_Secondary Th1_resting day 4-6 in IL-2	4.7
Adrenal gland	7.4	83222 CC NAT (ODO3868)	1.4	93572_Secondary Th2_resting day 4-6 in IL-2	3.5
Thyroid	6.8	83235 CC Mod Diff (ODO3920)	14.0	93571_Secondary Tr1_resting day 4-6 in IL-2	7.0
Salivary gland	2.5	83236 CC NAT (ODO3920)	13.9	93568_primary Th1_anti-CD28/anti-CD3	22.4
Pituitary gland	5.7	83237 CC Gr.2 ascend colon (ODO3921)	16.2	93569_primary Th2_anti-CD28/anti-CD3	16.3
Brain (fetal)	14.4	83238 CC NAT (ODO3921)	5.2	93570_primary Tr1_anti-CD28/anti-CD3	21.8
Brain (whole)	19.6	83241 CC from Partial Hepatectomy (ODO4309)	13.9	93565_primary Th1_resting dy 4-6 in IL-2	30.2
Brain (amygdala)	3.7	83242 Liver NAT (ODO4309)	12.7	93566_primary Th2_resting dy 4-6 in IL-2	14.4
Brain (cerebellum)	2.1	87472 Colon mets to lung (OD04451-01)	3.4	93567_primary Tr1_resting dy 4-6 in IL-2	7.4
Brain (hippocampus)	22.7	87473 Lung NAT (OD04451-02)	1.5	93351_CD45RA CD4 lymphocyte_anti-CD28/anti-CD3	7.6
Brain (thalamus)	7.4	Normal Prostate Clontech A+ 6546-1	1.0	93352_CD45RO CD4 lymphocyte_anti-CD28/anti-CD3	11.1
Cerebral Cortex	47.3	84140 Prostate Cancer (OD04410)	3.1	93251_CD8 Lymphocytes_anti-CD28/anti-CD3	9.6
Spinal cord	8.3	84141 Prostate NAT (OD04410)	10.6	93353_chronic CD8 Lymphocytes 2ry_resting dy 4-6 in IL-2	9.7
CNS ca. (glio/astro)U87-MG	19.9	87073 Prostate Cancer (OD04720-01)	9.7	93574_chronic CD8 Lymphocytes 2ry_activated CD3/CD28	6.2
CNS ca. (glio/astro) U-118-MG	57.0	87074 Prostate NAT (OD04720-02)	8.3	93354_CD4_none	6.4
CNS ca. (astro) SW1783	10.0	Normal Lung GENPAK 061010	36.6	93252_Secondary Th1/Th2/Tr1_anti-CD95 CH11	9.3
CNS ca.* (neuro; met)SK-N-AS	44.8	83239 Lung Met to Muscle (ODO4286)	11.7	93103_LAK cells_resting	11.0
CNS ca. (astro) SF-539	37.4	83240 Muscle NAT (ODO4286)	3.4	93788_LAK cells_IL-2	10.4
CNS ca. (astro) SNB-75	62.0	84136 Lung Malignant Cancer (OD03126)	15.1	93787_LAK cells_IL-2+IL-12	7.4

CNS ca. (glio) SNB-19	24.8	84137 Lung NAT (OD03126)	17.4	93789_LAK cells_IL-2+IFN gamma	11.6
CNS ca. (glio) U251	40.3	84871 Lung Cancer (OD04404)	5.0	93790_LAK cells_IL-2+ IL-18	13.3
CNS ca. (glio) SF-295	100.0	84872 Lung NAT (OD04404)	6.3	93104_LAK cells_PMA/ionomycin and IL-18	4.8
Heart	0.0	84875 Lung Cancer (OD04565)	3.2	93578_NK Cells IL-2_resting	6.2
Skeletal muscle	0.0	85950 Lung Cancer (OD04237-01)	15.8	93109_Mixed Lymphocyte Reaction_Two Way MLR	12.3
Bone marrow	33.7	85970 Lung NAT (OD04237-02)	10.5	93110_Mixed Lymphocyte Reaction_Two Way MLR	8.7
Thymus	12.4	83255 Ocular Mel Met to Liver (ODO4310)	5.9	93111_Mixed Lymphocyte Reaction_Two Way MLR	3.5
Spleen	21.3	83256 Liver NAT (ODO4310)	3.6	93112_Mononuclear Cells (PBMCs)_resting	4.5
Lymph node	13.4	84139 Melanoma Mets to Lung (OD04321)	10.6	93113_Mononuclear Cells (PBMCs)_PWM	21.2
Colorectal	38.2	84138 Lung NAT (OD04321)	10.6	93114_Mononuclear Cells (PBMCs)_PHA-L	8.9
Stomach	9.9	Normal Kidney GENPAK 061008	26.2	93249_Ramos (B cell)_none	100.0
Small intestine	17.9	83786 Kidney Ca, Nuclear grade 2 (OD04338)	22.2	93250_Ramos (B cell)_ionomycin	28.7
Colon ca.SW480	27.7	83787 Kidney NAT (OD04338)	11.7	93349_B lymphocytes_PWM	20.0
Colon ca.* (SW480 met)SW620	30.8	83788 Kidney Ca Nuclear grade 1/2 (OD04339)	45.1	93350_B lymphoytes_CD40L and IL-4	7.8
Colon ca.HT29	8.1	83789 Kidney NAT (OD04339)	14.8	92665_EOL-1 (Eosinophil)_dbcAMP differentiated	8.0
Colon ca.HCT-116	35.4	83790 Kidney Ca, Clear cell type (OD04340)	26.6	93248_EOL-1 (Eosinophil)_dbcAMP/PMAionomycin	3.8
Colon ca. CaCo-2	37.6	83791 Kidney NAT (OD04340)	10.4	93356_Dendritic Cells_none	6.8
83219 CC Well to Mod Diff (ODO3866)	17.8	83792 Kidney Ca, Nuclear grade 3 (OD04348)	2.4	93355_Dendritic Cells_LPS 100 ng/ml	3.3
Colon ca.HCC-2998	19.9	83793 Kidney NAT (OD04348)	18.8	93775_Dendritic Cells_anti-CD40	6.3
Gastric ca.* (liver met) NCI-N87	73.2	87474 Kidney Cancer (OD04622-01)	5.6	93774_Monocytes_resting	10.6
Bladder	43.2	87475 Kidney NAT (OD04622-03)	0.5	93776_Monocytes_LPS 50 ng/ml	3.5
Trachea	10.3	85973 Kidney Cancer (OD04450-01)	21.2	93581_Macrophages_resting	7.6
Kidney	9.2	85974 Kidney NAT (OD04450-03)	9.3	93582_Macrophages_LPS 100 ng/ml	3.9

Kidney (fetal)	0.0	Kidney Cancer Clontech 8120607	0.0	93098_HUVEC (Endothelial)_none	8.5
Renal ca.786-0	53.6	Kidney NAT Clontech 8120608	0.9	93099_HUVEC (Endothelial)_starved	17.9
Renal ca. A498	36.1	Kidney Cancer Clontech 8120613	0.0	93100_HUVEC (Endothelial)_IL-1b	6.0
Renal ca.RXF 393	31.6	Kidney NAT Clontech 8120614	0.9	93779_HUVEC (Endothelial)_IFN gamma	7.8
Renal ca.ACHN	21.6	Kidney Cancer Clontech 9010320	2.7	93102_HUVEC (Endothelial)_TNF alpha + IFN gamma	5.7
Renal ca.UO-31	28.7	Kidney NAT Clontech 9010321	5.0	93101_HUVEC (Endothelial)_TNF alpha + IL4	5.6
Renal ca.TK-10	7.0	Normal Uterus GENPAK 061018	5.3	93781_HUVEC (Endothelial)_IL-11	4.9
Liver	14.2	Uterus Cancer GENPAK 064011	9.0	93583_Lung Microvascular Endothelial Cells_none	4.9
Liver (fetal)	14.5	Normal Thyroid Clontech A+ 6570-1	3.4	93584_Lung Microvascular Endothelial Cells_TNFa (4 ng/ml) and IL1b (1 ng/ml)	4.9
Liver ca. (hepatoblast) HepG2	59.9	Thyroid Cancer GENPAK 064010	1.8	92662_Microvascular Dermal endothelium_none	8.6
Lung	17.8	Thyroid Cancer INVITROGEN A302152	3.6	92663_Microsvasular Dermal endothelium_TNFa (4 ng/ml) and IL1b (1 ng/ml)	6.0
Lung (fetal)	9.6	Thyroid NAT INVITROGEN A302153	4.9	93773_Bronchial epithelium_TNFa (4 ng/ml) and IL1b (1 ng/ml) **	0.9
Lung ca. (small cell) LX-1	70.2	Normal Breast GENPAK 061019	8.5	93347_Small Airway Epithelium_none	1.3
Lung ca. (small cell) NCI-H69	29.9	84877 Breast Cancer (OD04566)	1.5	93348_Small Airway Epithelium_TNFa (4 ng/ml) and IL1b (1 ng/ml)	13.2
Lung ca. (s.cell var.) SHP-77	3.9	85975 Breast Cancer (OD04590-01)	23.8	92668_Coronery Artery SMC_resting	3.4
Lung ca. (large cell)NCI-H460	2.0	85976 Breast Cancer Mets (OD04590-03)	24.5	92669_Coronery Artery SMC_TNFa (4 ng/ml) and IL1b (1 ng/ml)	2.0
Lung ca. (non-sm. cell) A549	28.5	87070 Breast Cancer Metastasis (OD04655-05)	12.9	93107_astrocytes_resting	4.7
Lung ca. (non-s.cell) NCI-H23	36.1	GENPAK Breast Cancer 064006	11.8	93108_astrocytes_TNFa (4 ng/ml) and IL1b (1 ng/ml)	1.9
Lung ca (non-s.cell) HOP-62	29.9	Breast Cancer Clontech 9100266	3.2	92666_KU-812 (Basophil)_resting	5.8
Lung ca. (non-s.cl) NCI-H522	17.2	Breast NAT Clontech 9100265	1.8	92667_KU-812 (Basophil)_PMA/ionoycin	12.0
Lung ca. (squam.) SW 900	63.7	Breast Cancer INVITROGEN A209073	11.0	93579_CCD1106 (Keratinocytes)_none	4.9
Lung ca.	10.0	Breast NAT	7.1	93580_CCD1106	0.3

(squam.) NCI-H596		INVITROGEN A2090734		(Keratinocytes)_TNFa and IFNg **	
Mammary gland	4.6	Normal Liver GENPAK 061009	8.8	93791_Liver Cirrhosis	1.8
Breast ca.* (pl. effusion) MCF-7	0.0	Liver Cancer GENPAK 064003	4.9	93792_Lupus Kidney	1.6
Breast ca.* (pl.ef) MDA-MB-231	38.7	Liver Cancer Research Genetics RNA 1025	1.0	93577_NCI-H292	11.1
Breast ca.* (pl. effusion) T47D	0.0	Liver Cancer Research Genetics RNA 1026	0.8	93358_NCI-H292_IL-4	12.2
Breast ca.BT-549	4.6	Paired Liver Cancer Tissue Research Genetics RNA 6004-T	3.0	93360_NCI-H292_IL-9	7.6
Breast ca.MDA-N	19.0	Paired Liver Tissue Research Genetics RNA 6004-N	7.3	93359_NCI-H292_IL-13	6.1
Ovary	1.7	Paired Liver Cancer Tissue Research Genetics RNA 6005-T	0.2	93357_NCI-H292_IFN gamma	5.8
Ovarian ca.OVCAR-3	4.8	Paired Liver Tissue Research Genetics RNA 6005-N	0.0	93777_HPAEC_-	6.8
Ovarian ca.OVCAR-4	0.0	Normal Bladder GENPAK 061001	19.8	93778_HPAEC_IL-1 beta/TNA alpha	5.4
Ovarian ca.OVCAR-5	39.0	Bladder Cancer Research Genetics RNA 1023	3.1	93254_Normal Human Lung Fibroblast_none	2.1
Ovarian ca.OVCAR-8	36.6	Bladder Cancer INVITROGEN A302173	9.9	93253_Normal Human Lung Fibroblast_TNFa (4 ng/ml) and IL-1b (1 ng/ml)	1.9
Ovarian ca.IGROV-1	0.0	87071 Bladder Cancer (OD04718-01)	6.6	93257_Normal Human Lung Fibroblast_IL-4	3.6
Ovarian ca.* (ascites) SK-OV-3	65.5	87072 Bladder Normal Adjacent (OD04718-03)	4.0	93256_Normal Human Lung Fibroblast_IL-9	3.3
Uterus	1.6	Normal Ovary Res. Gen.	0.3	93255_Normal Human Lung Fibroblast_IL-13	2.3
Plancenta	8.9	Ovarian Cancer GENPAK 064008	6.8	93258_Normal Human Lung Fibroblast_IFN gamma	2.9
Prostate	0.0	87492 Ovary Cancer (OD04768-07)	100.0	93106_Dermal Fibroblasts CCD1070_resting	5.6
Prostate ca.* (bone met)PC-3	9.2	87493 Ovary NAT (OD04768-08)	3.6	93361_Dermal Fibroblasts CCD1070_TNF alpha 4 ng/ml	17.4
Testis	29.5	Normal Stomach GENPAK 061017	8.6	93105_Dermal Fibroblasts CCD1070_IL-1 beta 1 ng/ml	3.8
Melanoma	14.3	NAT Stomach	0.7	93772_dermal fibroblast_IFN gamma	2.6

Hs688(A).T		Clontech 9060359			
Melanoma* (met) Hs688(B).T	22.9	Gastric Cancer Clontech 9060395	3.9	93771_dermal fibroblast_IL-4	3.4
Melanoma UACC-62	9.7	NAT Stomach Clontech 9060394	5.3	93259_IBD Colitis 1**	0.2
Melanoma M14	12.7	Gastric Cancer Clontech 9060397	13.2	93260_IBD Colitis 2	0.4
Melanoma LOX IMVI	4.5	NAT Stomach Clontech 9060396	1.1	93261_IBD Crohns	0.3
Melanoma* (met) SK-MEL-5	21.8	Gastric Cancer GENPAK 064005	23.0	735010_Colon_normal	3.3
Adipose	6.7			735019_Lung_none	3.9
				64028-1_Thymus_none	7.7
				64030-1_Kidney_none	21.8

Table 12 shows the taqman results of clone FCTR4 indicating overexpression in ovarian cancer as compared to Normal Adjacent Tissue (NAT). In addition, increased expression is demonstrated by ovarian tumor cell line suggesting that antibodies could be used to treat ovarian tumors.

Table 13: Primer Design for Probe Ag427 (FCTR5)

Primer	Sequences	Length	Start Po	SEQ ID NO
Forward	5'-GAGCTACAGGCAGCCTCGAGT-3'	21	443	32
Probe	TET-5'-TGGCCCAGCTGACCCTGCTCA-3'-TAMRA	21		33
Reverse	5'-GGCTACGTCAGTGGGTTTGG-3'	20	449	34

Table 14: Taqman results for FCTR5

Tissue_Name	Panel 1	Tissue_Name	Panel 4D
Endothelial cells	10.7	93768_Secondary Th1_anti-CD28/anti-CD3	15.9
Endothelial cells (treated)	15.2	93769_Secondary Th2_anti-CD28/anti-CD3	14.7
Pancreas	16.2	93770_Secondary Tr1_anti-CD28/anti-CD3	21.9
Pancreatic ca.CAPAN 2	10.5	93573_Secondary Th1_resting day 4-6 in IL-2	12.3
Adipose	45.1	93572_Secondary Th2_resting day 4-6 in IL-2	16.2
Adrenal gland	61.6	93571_Secondary Tr1_resting day 4-6 in IL-2	16.2
Thyroid	13.1	93568_primary Th1_anti-CD28/anti-CD3	13.9

Salavary gland	33.7	93569_primary Th2_anti-CD28/anti-CD3	14.6
Pituitary gland	15.8	93570_primary Tr1_anti-CD28/anti-CD3	26.2
Brain (fetal)	7.2	93565_primary Th1_resting dy 4-6 in IL-2	56.3
Brain (whole)	6.3	93566_primary Th2_resting dy 4-6 in IL-2	27.7
Brain (amygdala)	8.4	93567_primary Tr1_resting dy 4-6 in IL-2	31.6
Brain (cerebellum)	6.8	93351_CD45RA CD4 lymphocyte_anti-CD28/anti-CD3	12.1
Brain (hippocampus)	7.9	93352_CD45RO CD4 lymphocyte_anti-CD28/anti-CD3	17.1
Brain (substantia nigra)	9.5	93251_CD8 Lymphocytes_anti-CD28/anti-CD3	9.1
Brain (thalamus)	7.9	93353_chronic CD8 Lymphocytes 2ry_resting dy 4-6 in IL-2	13.4
Brain (hypothalamus)	23.0	93574_chronic CD8 Lymphocytes 2ry_activated CD3/CD28	9.2
Spinal cord	9.5	93354_CD4_none	7.6
CNS ca. (glio/astro)U87-MG	12.6	93252_Secondary Th1/Th2/Tr1_anti-CD95 CH11	20.2
CNS ca. (glio/astro)U-118-MG	11.6	93103_LAK cells_resting	57.0
CNS ca. (astro)SW1783	4.3	93788_LAK cells_IL-2	18.8
CNS ca.* (neuro; met)SK-N-AS	10.4	93787_LAK cells_IL-2+IL-12	14.2
CNS ca. (astro) SF-539	11.6	93789_LAK cells_IL-2+IFN gamma	20.9
CNS ca. (astro) SNB-75	4.4	93790_LAK cells_IL-2+ IL-18	14.8
CNS ca. (glio)SNB-19	31.6	93104_LAK cells_PMA/ionomycin and IL-18	12.9
CNS ca. (glio)U251	17.3	93578_NK Cells IL-2_resting	17.4
CNS ca. (glio)SF-295	20.9	93109_Mixed Lymphocyte Reaction_Two Way MLR	43.5
Heart	14.3	93110_Mixed Lymphocyte Reaction_Two Way MLR	19.3
Skeletal muscle	11.7	93111_Mixed Lymphocyte Reaction_Two Way MLR	12.6
Bone marrow	21.9	93112_Mononuclear Cells (PBMCs)_resting	8.7
Thymus	20.9	93113_Mononuclear Cells (PBMCs)_PWM	28.5
Spleen	23.8	93114_Mononuclear Cells (PBMCs)_PHA-L	26.2
Lymph node	24.2	93249_Ramos (B cell)_none	0.3
Colon (ascending)	17.2	93250_Ramos (B cell)_ionomycin	1.2
Stomach	11.1	93349_B lymphocytes_PWM	25.7
Small intestine	21.5	93350_B lymphoytes_CD40L and IL-4	13.0
Colon ca.SW480	12.2	92665_EOL-1 (Eosinophil)_dbcAMP differentiated	26.4
Colon ca.* (SW480 met)SW620	8.6	93248_EOL-1 (Eosinophil)_dbcAMP/PMAionomycin	11.4
Colon ca.HT29	16.2	93356_Dendritic Cells_none	40.3
Colon ca.HCT-116	8.1	93355_Dendritic Cells_LPS 100 ng/ml	33.0
Colon ca.CaCo-2	22.1	93775_Dendritic Cells_anti-CD40	20.5
Colon ca.HCT-15	18.6	93774_Monocytes_resting	23.3
Colon ca.HCC-2998	21.9	93776_Monocytes_LPS 50 ng/ml	6.9
Gastric ca.* (liver met) NCI-N87	42.9	93581_Macrophages_resting	14.7
Bladder	95.3	93582_Macrophages_LPS 100 ng/ml	64.6
Trachea	18.3	93098_HUVEC (Endothelial)_none	6.8
Kidney	25.7	93099_HUVEC (Endothelial)_starved	13.9
Kidney (fetal)	15.8	93100_HUVEC (Endothelial)_IL-1b	7.5
Renal ca.786-0	16.5	93779_HUVEC (Endothelial)_IFN gamma	27.7
Renal ca.A498	16.5	93102_HUVEC (Endothelial)_TNF alpha + IFN gamma	11.8
Renal ca.RXF 393	7.4	93101_HUVEC (Endothelial)_TNF alpha + IL4	6.7

Renal ca.ACHN	11.9	93781_HUVEC (Endothelial)_IL-11	10.4
Renal ca.UO-31	15.8	93583_Lung Microvascular Endothelial Cells_none	8.8
Renal ca.TK-10	28.7	93584_Lung Microvascular Endothelial Cells_TNFa (4 ng/ml) and IL1b (1 ng/ml)	8.6
Liver	100.0	92662_Microvascular Dermal endothelium_none	22.1
Liver (fetal)	81.8	92663_Microsvasular Dermal endothelium_TNFa (4 ng/ml) and IL1b (1 ng/ml)	18.7
Liver ca. (hepatoblast) HepG2	28.3	93773_Bronchial epithelium_TNFa (4 ng/ml) and IL1b (1 ng/ml) **	35.4
Lung	10.7	93347_Small Airway Epithelium_none	10.9
Lung (fetal)	10.9	93348_Small Airway Epithelium_TNFa (4 ng/ml) and IL1b (1 ng/ml)	50.0
Lung ca. (small cell) LX-1	24.3	92668_Coronery Artery SMC_resting	27.9
Lung ca. (small cell) NCI-H69	41.5	92669_Coronery Artery SMC_TNFa (4 ng/ml) and IL1b (1 ng/ml)	25.4
Lung ca. (s.cell var.) SHP-77	4.6	93107_astrocytes_resting	7.4
Lung ca. (large cell)NCI-H460	46.3	93108_astrocytes_TNFa (4 ng/ml) and IL1b (1 ng/ml)	10.7
Lung ca. (non-sm. cell) A549	45.4	92666_KU-812 (Basophil)_resting	3.2
Lung ca. (non-s.cell) NCI-H23	54.3	92667_KU-812 (Basophil)_PMA/ionoycin	6.7
Lung ca (non-s.cell) HOP-62	50.7	93579_CCD1106 (Keratinocytes)_none	12.2
Lung ca. (non-s.cl) NCI-H522	38.4	93580_CCD1106 (Keratinocytes)_TNFa and IFNg **	100.0
Lung ca. (squam.) SW 900	30.8	93791_Liver Cirrhosis	27.6
Lung ca. (squam.) NCI-H596	15.5	93792_Lupus Kidney	32.3
Mammary gland	65.5	93577_NCI-H292	77.4
Breast ca.* (pl. effusion) MCF-7	4.4	93358_NCI-H292_IL-4	70.2
Breast ca.* (pl.ef) MDA-MB-231	3.5	93360_NCI-H292_IL-9	54.3
Breast ca.* (pl. effusion)T47D	8.7	93359_NCI-H292_IL-13	47.0
Breast ca. BT-549	5.7	93357_NCI-H292_IFN gamma	52.9
Breast ca.MDA-N	16.6	93777_HPAEC_-	23.8
Ovary	20.5	93778_HPAEC_IL-1 beta/TNA alpha	21.5
Ovarian ca. OVCAR-3	21.6	93254_Normal Human Lung Fibroblast_none	49.3
Ovarian ca.OVCAR-4	8.3	93253_Normal Human Lung Fibroblast_TNFa (4 ng/ml) and IL-1b (1 ng/ml)	40.3
Ovarian ca.OVCAR-5	26.1	93257_Normal Human Lung Fibroblast_IL-4	48.3
Ovarian ca.OVCAR-8	48.0	93256_Normal Human Lung Fibroblast_IL-9	29.3
Ovarian ca.IGROV-1	9.3	93255_Normal Human Lung Fibroblast_IL-13	73.7
Ovarian ca.* (ascites)SK-OV-3	8.8	93258_Normal Human Lung Fibroblast_IFN gamma	66.9
Uterus	13.4	93106_Dermal Fibroblasts CCD1070_resting	20.2
Plancenta	9.4	93361_Dermal Fibroblasts CCD1070_TNF alpha 4 ng/ml	35.1
Prostate	21.3	93105_Dermal Fibroblasts CCD1070_IL-1 beta 1 ng/ml	15.0
Prostate ca.* (bone met)PC-3	17.7	93772_dermal fibroblast_IFN gamma	21.8
Testis	11.7	93771_dermal fibroblast_IL-4	21.2
Melanoma Hs688(A).T	9.0	93259_IBD Colitis 1**	8.8
Melanoma* (met) Hs688(B).T	12.9	93260_IBD Colitis 2	3.5
Melanoma UACC-62	12.4	93261_IBD Crohns	1.3

Melanoma M14	9.5	735010_Colon_normal	20.3
Melanoma LOX IMVI	8.1	735019_Lung_none	40.3
Melanoma* (met) SK-MEL-5	8.8	64028-1_Thymus_none	33.5
Melanoma SK-MEL-28	8.0	64030-1_Kidney_none	21.0

Taqman results in Table 14 show high expression of clone FCTR5 in bladder, liver and adrenal gland suggesting a possible role in the treatment of diseases involving these tissues.

Table 15: Primer Design for Probe Ag1541 (FCTR6)

Primer	Sequences	TM	Length	Start Pos.	SEQ ID NO
Forward	5'-AGAAGAACACCCCAGGGATATA-3'	58.8	22	1076	35
Probe	FAM-5'-CCTCGTTGGTGAACTACAACCTCTGG-3'-TAMRA	67.9	26	1100	36
Reverse	5'-CCTCTAGCTGGGTCACTTTCTC-3'	59.5	22	1129	37

TABLE 16: TAQMAN RESULTS FOR FCTR6 (PANEL 1D)

Tissue_Name	Panel 1D Run 1	Panel 1D Run 2
Liver adenocarcinoma	0.0	0.0
Heart (fetal)	0.0	0.0
Pancreas	0.0	0.0
Pancreatic ca.CAPAN 2	0.0	0.0
Adrenal gland	0.0	0.0
Thyroid	0.0	0.0
Salivary gland	0.0	0.0
Pituitary gland	0.0	0.0
Brain (fetal)	0.5	0.4
Brain (whole)	1.1	1.7
Brain (amygdala)	0.0	1.8
Brain (cerebellum)	0.6	1.9
Brain (hippocampus)	3.3	3.4
Brain (thalamus)	1.0	1.2
Cerebral Cortex	1.6	2.6
Spinal cord	2.5	0.4
CNS ca. (glio/astro)U87-MG	0.0	0.0
CNS ca. (glio/astro)U-118-MG	0.0	0.0
CNS ca. (astro)SW1783	0.0	0.0
CNS ca.* (neuro; met)SK-N-AS	0.0	0.0
CNS ca. (astro)SF-539	0.0	0.0
CNS ca. (astro) SNB-75	0.7	0.0
CNS ca. (glio)SNB-19	0.0	0.0
CNS ca. (glio)U251	0.0	0.0
CNS ca. (glio)SF-295	0.0	0.8
Heart	0.0	0.0
Skeletal muscle	0.0	0.0

Bone marrow	0.0	0.0
Thymus	0.0	0.0
Spleen	0.0	0.0
Lymph node	0.0	0.0
Colorectal	0.0	0.6
Stomach	1.9	0.0
Small intestine	0.0	1.0
Colon ca. SW480	0.0	0.0
Colon ca.* (SW480 met)SW620	0.0	0.0
Colon ca. HT29	0.0	0.0
Colon ca. HCT-116	0.6	0.4
Colon ca.CaCo-2	1.5	0.0
83219 CC Well to Mod Diff (ODO3866)	0.0	0.0
Colon ca.HCC-2998	0.0	0.0
Gastric ca.* (liver met) NCI-N87	1.2	0.0
Bladder	0.0	0.0
Trachea	0.0	0.4
Kidney	0.8	1.2
Kidney (fetal)	0.5	0.7
Renal ca.786-0	0.0	0.0
Renal ca.A498	0.0	0.0
Renal ca.RXF 393	0.0	0.0
Renal ca.ACHN	0.0	0.0
Renal ca. UO-31	0.0	0.0
Renal ca.TK-10	0.0	0.0
Liver	0.0	0.0
Liver (fetal)	0.2	0.0
Liver ca. (hepatoblast) HepG2	0.0	0.0
Lung	0.0	0.0
Lung (fetal)	0.0	0.0
Lung ca. (small cell) LX-1	1.7	2.3
Lung ca. (small cell)NCI-H69	0.0	0.0
Lung ca. (s.cell var.) SHP-77	1.3	2.5
Lung ca. (large cell)NCI-H460	0.0	0.0
Lung ca. (non-sm. cell) A549	0.0	0.0
Lung ca. (non-s.cell) NCI-H23	1.2	0.4
Lung ca (non-s.cell) HOP-62	0.0	0.0
Lung ca. (non-s.cl) NCI-H522	0.0	0.0
Lung ca. (squam.) SW 900	0.0	0.7
Lung ca. (squam.) NCI-H596	0.0	1.3
Mammary gland	0.0	1.5
Breast ca.* (pl. effusion) MCF-7	0.0	0.0
Breast ca.* (pl.ef) MDA-MB-231	5.8	0.5
Breast ca.* (pl. effusion) T47D	1.2	0.3
Breast ca. BT-549	0.5	0.0
Breast ca. MDA-N	0.0	0.0
Ovary	0.0	0.0
Ovarian ca. OVCAR-3	0.0	0.0
Ovarian ca.OVCAR-4	0.0	0.0
Ovarian ca.OVCAR-5	3.6	0.7
Ovarian ca.OVCAR-8	0.0	0.0
Ovarian ca.IGROV-1	0.0	0.0
Ovarian ca.* (ascites) SK-OV-3	0.0	0.0
Uterus	0.0	0.0
Plancenta	0.0	0.0
Prostate	0.0	0.7
Prostate ca.* (bone met)PC-3	0.0	0.0
Testis	100.0	100.0
Melanoma Hs688(A).T	0.0	0.0

Melanoma* (met) Hs688(B).T	0.0	0.0
Melanoma UACC-62	0.0	0.0
Melanoma M14	0.0	0.0
Melanoma LOX IMVI	0.0	0.0
Melanoma* (met)SK-MEL-5	0.0	0.0
Adipose	0.5	0.0

Table 17: Taqman Results for FCTR6 (Panel 2D)

Tissue_Name	Panel 2D Run 1	Run 2
Normal Colon GENPAK 061003	5.4	2.4
83219 CC Well to Mod Diff (ODO3866)	7.3	0.0
83220 CC NAT (ODO3866)	5.8	1.5
83221 CC Gr.2 rectosigmoid (ODO3868)	3.4	0.0
83222 CC NAT (ODO3868)	0.0	0.0
83235 CC Mod Diff (ODO3920)	11.0	1.4
83236 CC NAT (ODO3920)	0.0	0.0
83237 CC Gr.2 ascend colon (ODO3921)	6.2	2.5
83238 CC NAT (ODO3921)	10.2	0.0
83241 CC from Partial Hepatectomy (ODO4309)	3.6	0.0
83242 Liver NAT (ODO4309)	0.0	2.4
87472 Colon mets to lung (OD04451-01)	7.2	4.4
87473 Lung NAT (OD04451-02)	0.0	0.0
Normal Prostate Clontech A+ 6546-1	4.8	2.9
84140 Prostate Cancer (OD04410)	3.5	0.0
84141 Prostate NAT (OD04410)	3.4	0.0
87073 Prostate Cancer (OD04720-01)	9.0	8.5
87074 Prostate NAT (OD04720-02)	0.0	0.0
Normal Lung GENPAK 061010	17.7	6.5
83239 Lung Met to Muscle (ODO4286)	0.0	2.3
83240 Muscle NAT (ODO4286)	0.0	0.0
84136 Lung Malignant Cancer (OD03126)	6.5	5.7
84137 Lung NAT (OD03126)	0.0	0.0
84871 Lung Cancer (OD04404)	0.0	0.0
84872 Lung NAT (OD04404)	0.0	0.0
84875 Lung Cancer (OD04565)	0.0	0.0
85950 Lung Cancer (OD04237-01)	0.0	0.0
85970 Lung NAT (OD04237-02)	0.0	0.0
83255 Ocular Mel Met to Liver (ODO4310)	4.3	0.0
83256 Liver NAT (ODO4310)	0.0	0.0
84139 Melanoma Mets to Lung (OD04321)	0.0	0.0
84138 Lung NAT (OD04321)	0.0	0.0
Normal Kidney GENPAK 061008	28.1	39.2
83786 Kidney Ca, Nuclear grade 2 (OD04338)	0.0	3.0
83787 Kidney NAT (OD04338)	22.7	31.6
83788 Kidney Ca Nuclear grade 1/2 (OD04339)	0.0	3.1
83789 Kidney NAT (OD04339)	97.3	100.0
83790 Kidney Ca, Clear cell type (OD04340)	0.0	0.0
83791 Kidney NAT (OD04340)	100.0	34.4
83792 Kidney Ca, Nuclear grade 3 (OD04348)	2.0	4.9
83793 Kidney NAT (OD04348)	30.2	19.9
87474 Kidney Cancer (OD04622-01)	0.0	2.4
87475 Kidney NAT (OD04622-03)	8.4	7.2
85973 Kidney Cancer (OD04450-01)	0.0	0.0
85974 Kidney NAT (OD04450-03)	47.3	12.9

Kidney Cancer Clontech 8120607	0.0	0.0
Kidney NAT Clontech 8120608	0.0	0.0
Kidney Cancer Clontech 8120613	0.0	0.0
Kidney NAT Clontech 8120614	20.6	22.9
Kidney Cancer Clontech 9010320	0.0	0.0
Kidney NAT Clontech 9010321	3.4	26.4
Normal Uterus GENPAK 061018	0.0	0.0
Uterus Cancer GENPAK 064011	14.9	0.0
Normal Thyroid Clontech A+ 6570-1	0.0	0.0
Thyroid Cancer GENPAK 064010	0.0	0.0
Thyroid Cancer INVITROGEN A302152	0.0	0.0
Thyroid NAT INVITROGEN A302153	0.0	0.0
Normal Breast GENPAK 061019	5.2	3.5
84877 Breast Cancer (OD04566)	0.0	0.0
85975 Breast Cancer (OD04590-01)	0.0	0.0
85976 Breast Cancer Mets (OD04590-03)	0.0	0.0
87070 Breast Cancer Metastasis (OD04655-05)	0.0	0.0
GENPAK Breast Cancer 064006	0.0	2.5
Breast Cancer Clontech 9100266	6.2	0.0
Breast NAT Clontech 9100265	0.0	0.0
Breast Cancer INVITROGEN A209073	1.5	2.5
Breast NAT INVITROGEN A2090734	24.3	26.2
Normal Liver GENPAK 061009	10.5	2.7
Liver Cancer GENPAK 064003	5.9	1.7
Liver Cancer Research Genetics RNA 1025	21.6	11.0
Liver Cancer Research Genetics RNA 1026	0.0	0.0
Paired Liver Cancer Tissue Research Genetics RNA 6004-T	3.3	13.5
Paired Liver Tissue Research Genetics RNA 6004-N	3.2	1.4
Paired Liver Cancer Tissue Research Genetics RNA 6005-T	0.0	0.0
Paired Liver Tissue Research Genetics RNA 6005-N	0.0	0.0
Normal Bladder GENPAK 061001	0.0	0.0
Bladder Cancer Research Genetics RNA 1023	0.0	0.0
Bladder Cancer INVITROGEN A302173	4.6	2.3
87071 Bladder Cancer (OD04718-01)	17.9	11.4
87072 Bladder Normal Adjacent (OD04718-03)	0.0	0.0
Normal Ovary Res. Gen.	0.0	0.0
Ovarian Cancer GENPAK 064008	1.7	4.8
87492 Ovary Cancer (OD04768-07)	0.0	2.1
87493 Ovary NAT (OD04768-08)	0.0	0.0
Normal Stomach GENPAK 061017	3.3	2.9
NAT Stomach Clontech 9060359	0.0	0.0
Gastric Cancer Clontech 9060395	0.0	0.0
NAT Stomach Clontech 9060394	0.0	0.0
Gastric Cancer Clontech 9060397	0.0	0.0
NAT Stomach Clontech 9060396	0.0	0.0
Gastric Cancer GENPAK 064005	6.3	3.8

Table 18: Taqman Results for clone 27455183.0.19 (Panel 4D)

Tissue_Name	Panel 4D Run 1	Run 2
93768_Secondary Th1_anti-CD28/anti-CD3	0.0	0.0
93769_Secondary Th2_anti-CD28/anti-CD3	0.0	0.0
93770_Secondary Tr1_anti-CD28/anti-CD3	13.5	17.1
93573_Secondary Th1_resting day 4-6 in IL-2	0.0	0.0
93572_Secondary Th2_resting day 4-6 in IL-2	0.0	0.0

93571_Secondary Tr1_resting day 4-6 in IL-2	0.0	0.0
93568_primary Th1_anti-CD28/anti-CD3	0.0	0.0
93569_primary Th2_anti-CD28/anti-CD3	0.0	0.0
93570_primary Tr1_anti-CD28/anti-CD3	0.0	0.0
93565_primary Th1_resting dy 4-6 in IL-2	0.0	0.0
93566_primary Th2_resting dy 4-6 in IL-2	0.0	0.0
93567_primary Tr1_resting dy 4-6 in IL-2	0.0	0.0
93351_CD45RA CD4 lymphocyte_anti-CD28/anti-CD3	0.0	0.0
93352_CD45RO CD4 lymphocyte_anti-CD28/anti-CD3	0.0	0.0
93251_CD8 Lymphocytes_anti-CD28/anti-CD3	0.0	0.0
93353_chronic CD8 Lymphocytes 2ry_resting dy 4-6 in IL-2	0.0	0.0
93574_chronic CD8 Lymphocytes 2ry_activated CD3/CD28	0.0	0.0
93354_CD4_none	5.8	0.0
93252_Secondary Th1/Th2/Tr1_anti-CD95 CH11	0.0	0.0
93103_LAK cells_resting	0.0	0.0
93788_LAK cells_IL-2	0.0	0.0
93787_LAK cells_IL-2+IL-12	0.0	0.0
93789_LAK cells_IL-2+IFN gamma	0.0	0.0
93790_LAK cells_IL-2+ IL-18	0.0	0.0
93104_LAK cells_PMA/ionomycin and IL-18	0.0	0.0
93578_NK Cells IL-2_resting	0.0	0.0
93109_Mixed Lymphocyte Reaction_Two Way MLR	0.0	0.0
93110_Mixed Lymphocyte Reaction_Two Way MLR	0.0	0.0
93111_Mixed Lymphocyte Reaction_Two Way MLR	0.0	0.0
93112_Mononuclear Cells (PBMCs)_resting	0.0	0.0
93113_Mononuclear Cells (PBMCs)_PWM	0.0	0.0
93114_Mononuclear Cells (PBMCs)_PHA-L	0.0	0.0
93249_Ramos (B cell)_none	0.0	38.2
93250_Ramos (B cell)_ionomycin	0.0	0.0
93349_B lymphocytes_PWM	0.0	68.8
93350_B lymphoytes_CD40L and IL-4	31.0	0.0
92665_EOL-1 (Eosinophil)_dbcAMP differentiated	0.0	0.0
93248_EOL-1 (Eosinophil)_dbcAMP/PMAionomycin	0.0	0.0
93356_Dendritic Cells_none	0.0	0.0
93355_Dendritic Cells_LPS 100 ng/ml	0.0	0.0
93775_Dendritic Cells_anti-CD40	32.5	0.0
93774_Monocytes_resting	0.0	0.0
93776_Monocytes_LPS 50 ng/ml	0.0	0.0
93581_Macrophages_resting	0.0	0.0
93582_Macrophages_LPS 100 ng/ml	0.0	0.0
93098_HUVEC (Endothelial)_none	0.0	0.0
93099_HUVEC (Endothelial)_starved	11.3	0.0
93100_HUVEC (Endothelial)_IL-1b	0.0	14.6
93779_HUVEC (Endothelial)_IFN gamma	0.0	0.0
93102_HUVEC (Endothelial)_TNF alpha + IFN gamma	0.0	0.0
93101_HUVEC (Endothelial)_TNF alpha + IL4	0.0	0.0
93781_HUVEC (Endothelial)_IL-11	0.0	0.0
93583_Lung Microvascular Endothelial Cells_none	0.0	0.0
93584_Lung Microvascular Endothelial Cells_TNFa (4 ng/ml) and IL1b (1 ng/ml)	0.0	0.0
92662_Microvascular Dermal endothelium_none	0.0	0.0
92663_Microsvasular Dermal endothelium_TNFa (4 ng/ml) and IL1b (1 ng/ml)	0.0	0.0
93773_Bronchial epithelium_TNFa (4 ng/ml) and IL1b (1 ng/ml) **	0.0	0.0
93347_Small Airway Epithelium_none	0.0	0.0
93348_Small Airway Epithelium_TNFa (4 ng/ml) and IL1b (1 ng/ml)	0.0	0.0
92668_Coronery Artery SMC_resting	0.0	0.0
92669_Coronery Artery SMC_TNFa (4 ng/ml) and IL1b (1 ng/ml)	0.0	0.0
93107_astrocytes_resting	0.0	0.0

93108_astrocytes_TNFa (4 ng/ml) and IL1b (1 ng/ml)	0.0	0.0
92666_KU-812 (Basophil)_resting	0.0	40.3
92667_KU-812 (Basophil)_PMA/ionoycin	0.0	0.0
93579_CCD1106 (Keratinocytes)_none	0.0	0.0
93580_CCD1106 (Keratinocytes)_TNFa and IFNg **	0.0	0.0
93791_Liver Cirrhosis	100.0	99.3
93792_Lupus Kidney	0.0	0.0
93577_NCI-H292	0.0	0.0
93358_NCI-H292_IL-4	0.0	0.0
93360_NCI-H292_IL-9	10.6	0.0
93359_NCI-H292_IL-13	0.0	65.5
93357_NCI-H292_IFN gamma	0.0	24.8
93777_HPAEC_-	0.0	0.0
93778_HPAEC_IL-1 beta/TNA alpha	0.0	0.0
93254_Normal Human Lung Fibroblast_none	0.0	0.0
93253_Normal Human Lung Fibroblast_TNFa (4 ng/ml) and IL-1b (1 ng/ml)	0.0	0.0
93257_Normal Human Lung Fibroblast_IL-4	0.0	0.0
93256_Normal Human Lung Fibroblast_IL-9	0.0	0.0
93255_Normal Human Lung Fibroblast_IL-13	0.0	0.0
93258_Normal Human Lung Fibroblast_IFN gamma	0.0	0.0
93106_Dermal Fibroblasts CCD1070_resting	0.0	0.0
93361_Dermal Fibroblasts CCD1070_TNF alpha 4 ng/ml	0.0	43.8
93105_Dermal Fibroblasts CCD1070_IL-1 beta 1 ng/ml	0.0	0.0
93772_dermal fibroblast_IFN gamma	42.0	27.7
93771_dermal fibroblast_IL-4	10.7	90.1
93259_IBD Colitis 1**	0.0	0.0
93260_IBD Colitis 2	13.8	0.0
93261_IBD Crohns	0.0	46.7
735010_Colon_normal	15.6	0.0
735019_Lung_none	12.9	16.8
64028-1_Thymus_none	69.3	100.0
64030-1_Kidney_none	0.0	0.0

Taqman results in Table 18 demonstrate that clone FCTR6 is differentially expressed in clear cell Renal cell carcinoma tissues versus the normal adjacent kidney tissues and thus could have a potential role in the treatment of renal cell carcinoma.

EQUIVALENTS

Although particular embodiments have been disclosed herein in detail, this has been done by way of example for purposes of illustration only, and is not intended to be limiting with respect to the scope of the appended claims which follow. In particular, it is contemplated by the inventors that various substitutions, alterations, and modifications may be made to the invention without departing from the spirit and scope of the invention as defined by the claims. The choice of nucleic acid starting material, clone of interest, or library type is believed to be a matter of routine for a person of ordinary skill in the art with knowledge of the embodiments described

herein. Other aspects, advantages, and modifications considered to be within the scope of the following claims.